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The fine structure of nuclear inclusions

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INTRODUCTION

The fine structure of spherical inclusions was first described by de Thé (1960) and later defined under the name of nuclear bodies by Weber & Frommes (1963) and Weber, Whipp, Usenik & Frommes (1964). Since then they have been observed in pathological (Bouteille, Kalifat & Delarue, 1967: Krishan, Uzman & Hedley-Whyte, 1967; Büttner & Horstmann, 1967) and normal (Horstmann, Richter & Rosen-Runge, 1966; Kjærheim, 1968*a*) cells. Nuclear bodies have a characteristic spherical appearance, varying in size from 0·5 to 1·5 μm in diameter, and are sharply delineated from the surrounding karyoplasm, although they are not surrounded by a limiting membrane. As a rule, they bear no obvious relationship to the nucleolus or the nuclear membrane. In the monkey nuclear bodies have been demonstrated in the epithelium of the kidney (Büttner & Horstmann, 1967), in the rat, in the lymphocyte (Büttner & Horstmann, 1967) and in the domestic fowl only in the adrenocortical cells (Kjærheim, 1968*a*). Both Weber (1964 *et al.*) and Kjærheim (1968*b*) have found them to increase in number after ACTH stimulation.

Intranuclear fibrillar bundles have also been described in normal (Siegesmund, Dutta & Fox, 1964; Mugnaini, 1967; Popoff & Stewart, 1968) and pathological (Granboulan, Tournier, Wicher & Bernhard, 1963; Chandler & Willis, 1966; Masurovsky, Benitez & Murray, 1967; Périer, Vanderhaegen & Pelc, 1967) cells. Usually they appear as rod-shaped bundles of closely packed parallel fibrils of about 550 μm in length and 80 μm in width (Popoff & Stewart, 1968). In the monkey fibrillar bundles have been demonstrated in the neurons (Siegesmund *et al.* 1964) and in the rat they have been found in neurons (Chandler, 1965, 1966) and ependymal cells (Hirano & Zimmerman, 1967). In the domestic fowl they have, to my knowledge, not been demonstrated previously.

In order to decide whether or not these two types of nuclear inclusions are normal organelles, cells from different organs of the domestic fowl, the rat and the monkey, all apparently healthy animals, were systematically examined for these particular structures (Table 1).

MATERIALS AND METHODS

Ten white hens about 1½ years of age, three chickens about 3 months old, three adult male rats and one adult male rhesus monkey were used in this investigation. Two of the hens received daily intramuscular injections of 15 i.u. ACTH for 5 d, and two other hens received the same dose for 10 d.

Fixation of tissues of the hens, chickens and rats was performed by intracardial perfusion of dextran under Nembutal anaesthesia, followed by 1·7 % glutaraldehyde in 0·1 M phosphate buffer at pH 7·3. Tissue blocks from different organs were then fixed separately in glutaraldehyde for an additional period of 2–24 h. Subsequently the tissue blocks were rinsed for 30–60 min in 0·15 M phosphate buffer at pH 7·3

Table 1. *Review of the different cell types where nuclear bodies were found*

		Domestic fowl	Rat	Monkey
Spleen	Lymphocytes	+	·	·
	Retic. cells	+	·	·
	Endothelium	+	·	·
Lungs	Endothelium	+	·	·
	Mesothelium	+	·	·
Liver	Liver cells	+	·	·
	Endothelium	+	·	·
Pancreas	Exocrine cells	+	·	·
	Endothelium	+	·	·
Large int.	Epithelium	+	·	·
	Endothelium	+	·	·
	Smooth muscle	+	·	·
Small int.	Epithelium	+	·	·
	Endothelium	+	·	·
	Smooth muscle	+	·	·
Ovary	Epithelium	+	·	·
	Stroma cells	+	·	·
	Theca cells	+	·	·
	Endothelium	+	·	·
Adrenal gld.	Endothelium	+	·	·
	Cortex cells	+	·	·
Brain	Neurons	+	+	+
	Astrocytes	+	+	+
	Oligodendrocytes	+	+	·
	Schwann cells	+	·	·
	Endothelium	+	·	+
	Ependymals cells	+	·	·
	Choroid plexus	+	·	·
	Endothelium	+	·	·
Kidney	Epithelium	+	+	·
	Endothelium	+	+	·
Blood vessels	Endothelium	+	+	+
	Smooth muscle	+	+	+

and postfixed in 2 % osmium tetroxide at 4 °C for 2 h. The blocks were dehydrated in graded series of acetone and embedded in Vestopal W. Ultrathin sections were cut on an LKB Ultratome III and treated with uranyl acetate (3 %) for 30 min followed by lead citrate for 5 min. The sections were examined in a Siemens Elmiskop Ia electron microscope equipped with 50 μm platinum apertures.

Fixation of tissues of the monkey was by intracardial perfusion of 4 % unbuffered formaldehyde. The subsequent procedures were identical with the ones described for the other animals. The organs examined are listed in Table 1.

RESULTS

Despite a certain variability in size and ultrastructural appearance, three general types of nuclear bodies could be recognized in the domestic fowl, one type in the rat, and one type in the monkey. The search for different types was, however, not so extensive in the last two species as the nuclear bodies observed in the rat, and in the monkey were found to be morphologically almost identical with type I in the fowl.

Type 1 (Figs. 1, 2). This type comprises nuclear bodies with a diameter of 0·2–0·4 μm. They consist of an outer capsule of fibrils and a homogeneous and less dense core. In sections where the nuclear bodies were cut tangentially, no core could be seen. Some sections revealed that the bodies had an elongated form, and indicated that they can have a tubular shape with closed ends. Sometimes the cores seemed to be membrane-bound and they were often surrounded by a halo of low and variable electron density. This type was most widely distributed, and was observed in every organ and cell type examined in all the animals.

Type 2 (Figs. 3, 4). This type comprises nuclear bodies with a diameter of 0·3–0·5 μm. They had an outer smooth membrane, the surface of which was surrounded by a zone of fluffy material of variable thickness. The inside of this type of nuclear body consisted of fairly homogeneous or finely granular matrix. Sometimes an additional membrane-bound component was found inside this matrix and a small dense body could sometimes be observed within the matrix.

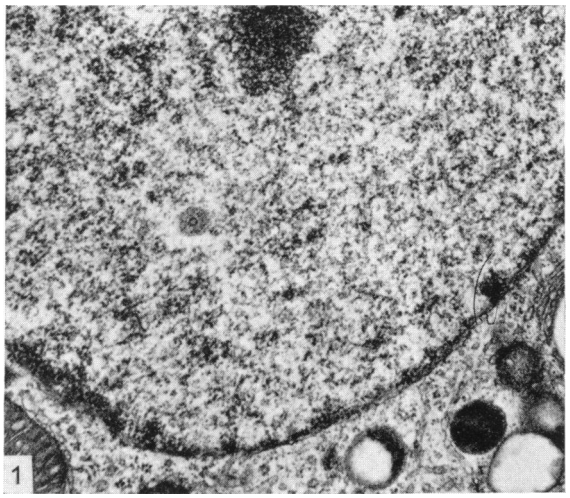
Type 3 (Figs. 5, 6). This type was surrounded by a dense osmiophilic layer surrounded by a lipid-like vacuole, varying in diameter from 0·3 to 0·7 μm. The outer surface of the dense layer was covered by a zone of fluffy material like the one surrounding type 2 nuclear bodies. In some instances this type of nuclear body was found in groups of three or more, and they were surrounded by a common mass of fluffy material.

In addition to these three general types, other forms were regularly encountered. They all had, however, one or more characteristics in common with the type described, suggesting that they were transitional forms (Figs. 7, 8).

It was impossible to obtain exact quantitative data of the number of bodies on account of the enormous number of cells and organs examined in the electron microscope. Since intermediate forms have also been regularly observed, it cannot be ruled out that the different types possibly represent different stages in the genesis or breakdown of a single type of body.

There did not seem to be any morphological difference between the nuclear bodies observed in the chicken and the hen or between the ones observed in the ACTH-treated and untreated hens. However, ACTH seemed to increase the frequency and degree of the differentiation of nuclear bodies as they were more often observed in the treated animals.

Intranuclear fibrillary bundles (Fig. 9). Single, rod-shaped bundles varying in length from 3 to 0·3 μm and in width from 0·3 to 0·1 μm, depending on the cutting angle, were found in cortical neurons, cells of exocrine pancreas and reticulum cells of the spleen in the hen. The inclusions consisted of closely packed parallel fibrils. In the neurons as well as the reticulum cells fibrillary bundles and nuclear bodies were found within the same nucleus and at a small distance from each other.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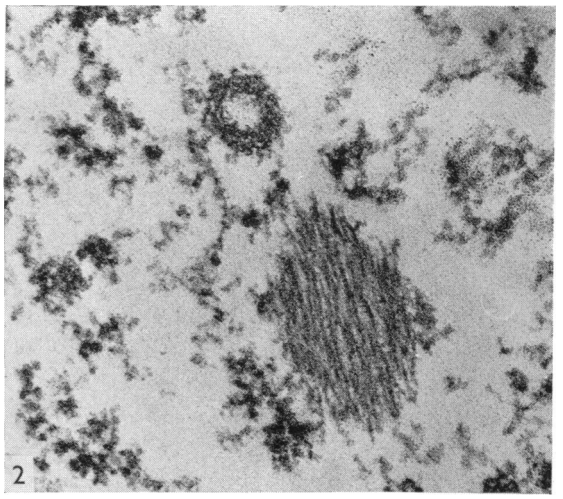
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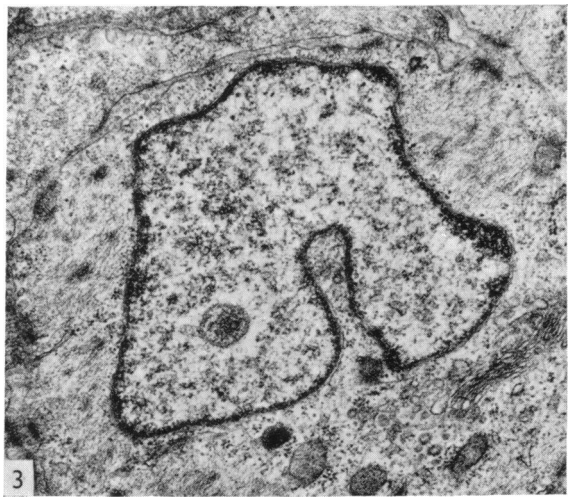
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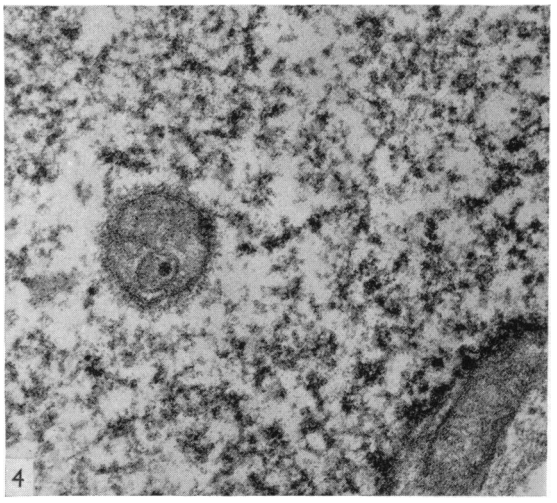
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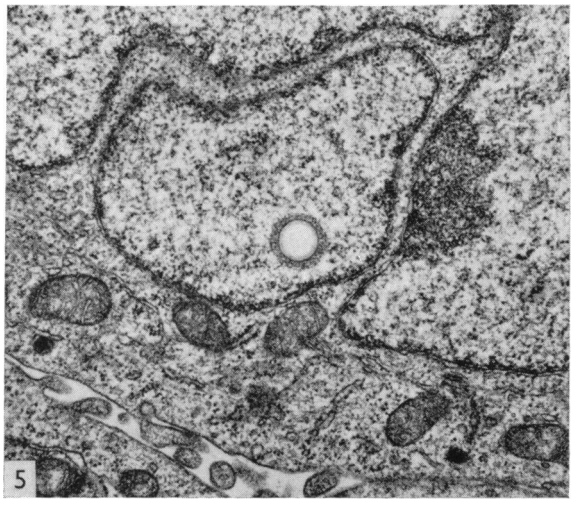
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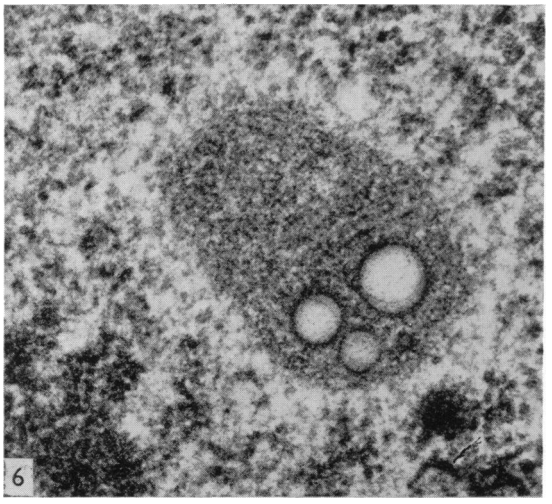
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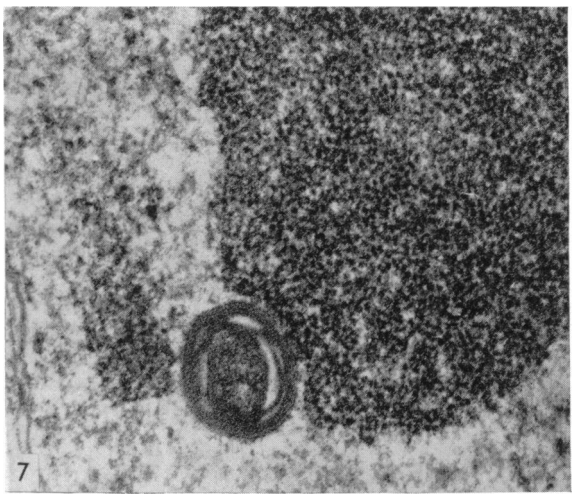

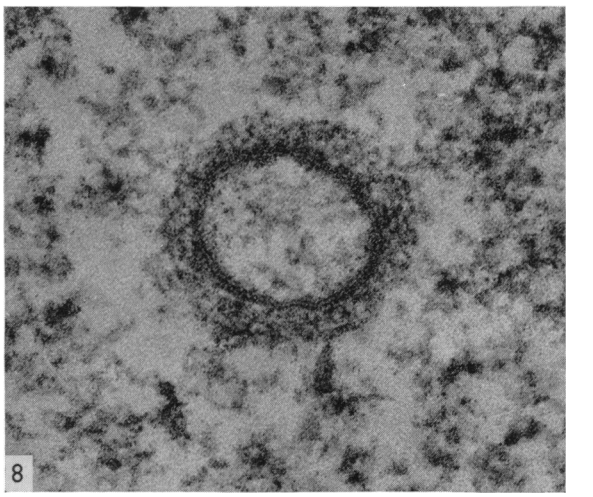

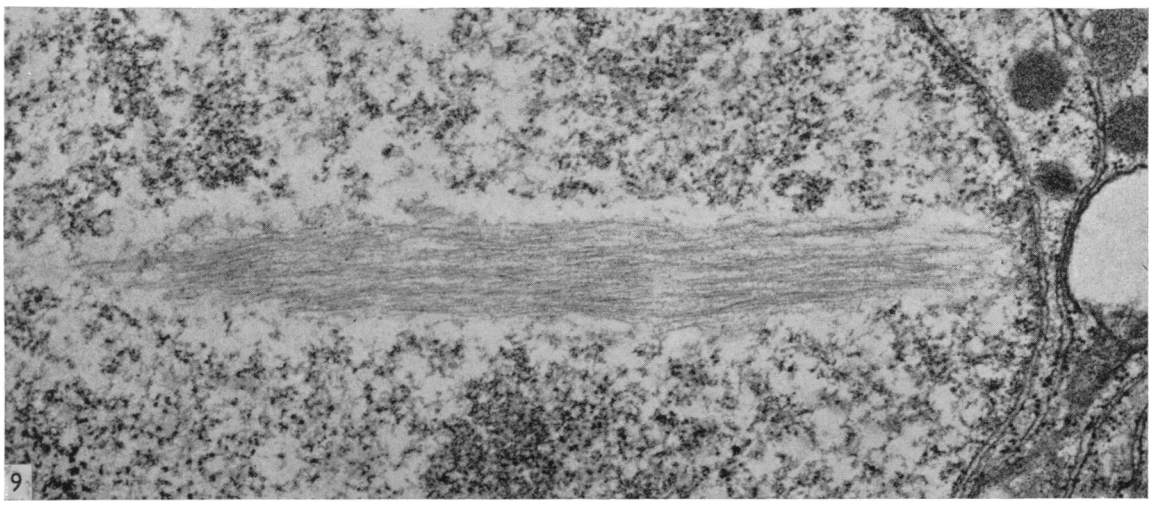

Fig. 7. Nuclear body of transitional type from stroma cell of ovary of the hen. × 60000.

Fig. 8. Nuclear body of transitional type from smooth muscle of the hen. × 120000.

Fig. 9. Fibrillary bundles from the exocrine cell of pancreas of the hen. × 32000.

Figs. 1, 3 and 5 represent the three different general types seen at a magnification of × 24 000, and 2, 4 and 6 the same types at a higher magnification (× 60 000).

Fig. 1. Nuclear body of type 1 from a stroma cell of chicken ovary.

Fig. 2. Nuclear body of type 1 and fibrillary bundles from a neuron of the hen.

Fig. 3. Nuclear body of type 2 from smooth muscle of chicken ovary.

Fig. 4. Nuclear body of type 2 from smooth muscle of chicken ovary with the rather complex membrane components and dense body.

Fig. 5. Nuclear body of type 3 from epithelial cell of chicken ovary.

Fig. 6. Nuclear body of type 3 from steroid-producing cell of chicken ovary.

However, no further relationship could be discerned between these two forms of inclusions. In the pancreas large fibrillary bundles were observed in the absence of nuclear bodies.

DISCUSSION

Since nuclear bodies have so far been observed mainly in pathological conditions, it has been considered that their occurrence may be related to such conditions, or even to their etiology. Robertson & MacLean (1965) observed nuclear bodies in gliomas, Krishan *et al.* (1967) in leukemia, and Bouteille *et al.* (1967) in a survey of the literature found the majority of the reported cases to be in tumours. Their own investigation revealed that they occurred also in viral diseases, as also reported by others (Popoff & Stewart, 1968). Weber *et al.* (1964), however, considered them to be normal organelles in the adrenal gland of the calf, and this view was supported by Kjærheim (1968*a*) concerning the adrenal cortex of the hen.

The present study deals with apparently healthy, normal animals, and only four hens received ACTH-injections. Several different organs and cell types within the same animal were examined and nuclear bodies were demonstrated in all. It is therefore reasonable to presume that nuclear bodies are normal organelles, probably existing in all cell types, and the existence of such structures in pathological tissue does not justify the suggestions that they may have a pathological or pathognomonic significance.

The frequency of these nuclear organelles is rather difficult to estimate. Nuclear bodies of type I were most often encountered. Horstmann (1965), in human epididymis, demonstrated a close relationship between differentiation of nuclear bodies and maturation of epididymal cells. Weber *et al.* (1964) and Kjærheim (1968*b*) found them to differentiate and increase in number following ACTH stimulation, and this was confirmed in the present study. These observations indicate that the differentiation and frequency of these nuclear inclusions may be under the influence of hormones. Certain cell types, particularly reticuloendothelial cells, seem to have a higher number of nuclear bodies than others (Bouteille *et al.* 1967), and in the present study they appeared to be especially common in cells of the pancreas, suprarenal, ovary and endothelium. So far, however, no definite conclusions can be drawn about their frequency. The different types of nuclear bodies observed in the present study seem to accord with previous descriptions, although certain differences exist, which may partly be accounted for by the different fixation methods used, and partly by differences in the species examined. Bouteille *et al.* (1967) described five types, Büttner & Horstmann (1967) four, and Popoff & Stewart (1968) three types. In this investigation three types could be distinguished in the fowl, but the presence of numerous intermediate forms indicates a certain morphological entity within the same species, and, when compared with nuclear bodies previously described, also from one species to another.

Krishan *et al.* (1967), on the basis of histochemical staining and enzyme extractions, suggested that fibrous nuclear bodies do not contain any DNA or RNA, but may contain protein. As pointed out by Krishan *et al.* (1967) and Bouteille *et al.* (1967), however, their number and size in a cell are dependent in some way on an altered or special metabolic state in the cell nucleus, or on cellular hyperactivity, the

cause of which may be physiological, hormonal, drug-induced or tumour-dependent. The function of these nuclear inclusions has not, however, been established.

Intranuclear fibrillary bundles were first observed in the light microscope by several anatomists (Mann, 1894; Roncoroni, 1895; Holmgren, 1899). Cajal (1910) referred to them as 'rodlets of Roncoroni' and Siegesmund *et al.* (1964) have confirmed Cajal's work by demonstrating them in neurons, using the electron microscope. Since then they have been observed in neurons and ependymal cells of the normal rat (Chandler, 1965, 1966) and neurons and glia cells of the Atlantic hagfish (Mugnaini, 1967). They have also been demonstrated within neurons in pathological conditions, such as tumours (Robertson & MacLean, 1965) and subacute sclerosing leukoencephalitis (Périer, 1967). Based on his own work and an analysis of the literature, Mugnaini (1967) suggests that non-crystalline filamentous inclusions are a rather unspecific phenomenon governed by metabolic conditions as yet poorly understood, and may be related to pathological as well as physiological conditions of the cell. Popoff & Stewart (1968) did not find any connexion between fibrillary bundles and nuclear bodies, and they concluded that there is no clear relationship between the two types of structure; Masurovsky *et al.* (1967), however, suggested that nuclear bodies may be involved in the elaboration of fibrillary bundles. In the present study nuclear bodies were widely and regularly encountered, while fibrillary bundles were rare. Both of these nuclear inclusions were observed as single independent structures, and there seems to be little evidence that there should be any relationship between them.

This investigation, together with previous reports, thus indicates that nuclear bodies are normal cell organelles, able to change their morphology with the functional state of the cell. There is no evidence that they should be an etiological factor in pathological conditions.

The fibrillary bundles seem to be an unspecific phenomenon within the normal range of biological variation without any specific relationship to the nuclear bodies.

SUMMARY

Nuclear bodies were observed within many different organs and cell types of normal chickens, hens, rats and a monkey. An arbitrary classification of nuclear bodies into three general types has been proposed, but several transitional types were also observed.

The study reveals the nuclear bodies to be normal organelles of the cell without any pathological or etiological significance. The increase of nuclear bodies seen after ACTH-injections is indicative of an altered activity of the cell. Fibrillary bundles were rather seldom encountered; their presence may be regarded as a normal biological variation within the nucleus.

No relationship between these two different nuclear inclusions could be demonstrated.

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