Intranuclear inclusions in experimental yellow fever

By C. MAGARINOS TORRES.

Examining (1) liver sections from forty-three *Macacus rhesus* inoculated with yellow fever virus (Brazilian (2) and African (3) strains) I found acidophilic intranuclear inclusions (figs. *f*, *g*, *h*, *i*, *j* and *k*) in thirty-one of them.

They were observed still in two injected *M. cynomolgus*, animals whose receptiveness to yellow fever virus was first demonstrated by ARAGÃO (Brasil-Medico, July 7, 1928).

The inclusions were not evidenced, on the other hand, in the liver from six uninoculated *rhesus* monkeys as well as from nine *rhesus* injected with miscellaneous material, *e. g.*, liver from yellow fever patient, NOGUCHI's medium inoculated with virus (several passages), *Bacillus typhosus* culture, etc..

Plate 1 represents besides acidophilic intranuclear inclusions just mentioned, non specific nuclear changes (figs. *b*, *c*, *d* and *e*) dependent on the necrobiosis of the liver cell and which were observed in injected as well as in uninoculated animals. Special reference to them is made here on account of the presence inside the nucleus of eosin-staining substances.

Such cytological minutiae shall be appreciated in well preserved tissues and well stained sections; the study of frozen sections, except in animals where inclusions appeared very abundant, has given untrusty results and it was found that postmortem changes render specially difficult the recognition of inclusions when scarce.

Fig. *a* represents two normal liver cells from uninoculated *rhesus*. The nuclei measure respectively 6 μ 7 by 6 μ 7 and 7 μ by 6 μ 7, the dimensions being the average ones, as rather larger nuclei are at times to be found in some specimens. Chromatin granules appear larger and more numerous at the vicinity of the nucleoli and of the nuclear membrane. No other eosin-staining substance beyond the excentric true nucleolus appears inside the nucleus of a normal liver cell of the *rhesus*.

The nuclei in figs. *f* and *g* is somewhat enlarged as compared with fig. *a*, measuring respectively 7 μ 6 by 7 μ 9, and 8 μ by 7 μ 6, showing as characteristic change the acidophilic mass which is single, presents a semilunar shape and lies at the centre, being well distinct from the nucleolus and separated from the nuclear membrane by a clear area. In early stages of oxychromatic degeneration (figs. *f*, *g* and *h*) chromatin granules

---

(1) By courtesy of Drs. H. de BEAUREPAIRE ARAGÃO, A. MARQUES DA CUNHA and J. MUNIZ.

(2) From different sources (yellow fever patients at Rio de Janeiro).

(3) Virus sent from New York to B. ARAGÃO.

somewhat smaller than in normal nucleus occur; they become entirely absent, however, in later stages (figs. *i*, *j* and *k*) when an empty , structureless space surrounds the acidophilic inclusion, the only substance demonstrable inside the nucleus.

Fig. *h* represents a "butterfly-like" intranuclear inclusion, a frequent and characteristic picture in monkeys injected with the Brazilian strains (several sources); therefore I was not able to find such stages in the few animals I examined of the African strain series. With regard to the absence of nucleolus in this figure, the plane of sectioning accounts probably for it.

In figs. *i*, *j* and *k* the nuclei are distinctly enlarged measuring respectively 9 μ 5 by 7 μ, 8 μ by 7 μ 6 and 11 μ 4 by 8 μ 5. Nucleolar substances staining dark violet are to be seen either inside the acidophilic inclusions themselves (figs. *i* and *j*) or in their vicinity (fig. *k*); the basichromatin disappears from the inside of the nucleus, condensing itself upon the nuclear membrane where it forms rod-like structures, sometimes separated by a very thin strand of pinkish material which marks the outline of the nucleus.

Moreover, the inclusions are not homogeneous and hyaline in structure but appear more or less granular (figs. *j* and *k*); the more compact the inclusion is, the more intense is its coloration by eosin.

Of course, all liver cells represented in the Plate show fat vacuoles at the cytoplasm besides oxychromatic degeneration.

Figs. *b*, *c*, *d* and *e* represent early stages in necrobiosis of liver cell. Inside the nucleus stained uniformly and at times a deep blue, besides a violet stained granule (nucleolus?) and eosin-staining material, no chromatin granule is visible (*chromatolysis*). The eosin-staining substances form sometimes a ring around the basophilic granule (figs. *d* and *e*); in figs. *b* and *c* the acidophilic material is somewhat diffuse and indistinct.

As a rule, such nuclei are not enlarged, measuring in average 7 μ 9 by 6 μ 6 and although containing acidophilic masses, are sharply distinguishable from those presenting oxychromatic degeneration by the shape and appearance of the eosin-staining substance as compared with the nuclear inclusions, in addition to the diffuse deep blue staining and chromatolysis of the nucleus.

The yellow fever inclusions are confined strictly to the interior of the nucleus, figs. *f*, *g* and *k* showing that they are distinct from the nucleolus, a fact better evidenced in preparations stained with carbol-fuchsin and counter-stained with LOEFFLER's methylene blue and differentiated in alcohol. The nucleoli may appear intensely red in such preparations, while the yellow fever bodies appear blue or blue-violet.

The material composing the inclusions is readily distinguishable from nuclear chromatin in liver sections submitted to FEULGEN's Nuclealfärbung (4); in them, while the chromatin staining blue is found deposited upon the nuclear membrane, the yellow fever bodies remain unstained.

As is the case with herpes, it seems evident that the material which constitutes the yellow fever "inclusions" may partially at least be compo-

---

(4) FEULGEN, A. Die Nuclealfärbung in Abderhalden: Handb. d. biol. Arbeitsmeth., Lief. 213, 1926, S. 1055 u. 1071.

C. MAGARINOS TORRES — Intranuclear inclusions in experimental yellow fever (Brazilian strain).

A. Leal del

Liver cells of *M. rhesus*. Helly's fixative ; hematoxylin-eosin stain ; Zeiss 1/12 im. and oc. comp. 6.

Fig. *a* represent a normal liver cell.

Figs. *b—e* show necrobiotic (non-specific) nuclear changes.

Figs. *f—k* represent characteristic nuclear changes; the inclusions are represented by the pink intranuclear masses.

Fig. *h* represents a "butterfly-like" body.

Figs. *i*, *j* and *k* show the end stages of oxychromatic nuclear degeneration, as well as increase in the size of the nucleus (specially in *k*).

Figs. *f*, *g*, and *i* show acidophilic mass independent of nucleolus.

Typ. do Inst. Osw. Cruz

EXPLANATION OF PHOTOMICROGRAPHS

Figs. 1, 2, 3, 4, and 5 are photomicrographs, magnification 540 diameters, of liver sections from *rhesus* monkeys. HELLY's fixative; hematoxylin and eosin stain.

Fig. 1 represents normal structure of the nucleus from uninoculated *rhesus*. Some liver cells show fatty degeneration.

Figs. 2 and 3 represent acidophilic intranuclear inclusions at almost every liver cells from *rhesus* injected with yellow fever virus (Brazilian strain). "Entamoeba-like" and "butterfly-like" intranuclear inclusions are a common picture, the "granular stage" being less frequently found. Note in Fig. 2 two typical "butterfly-like" bodies. These figures also show absence of necrotic liver cells.

Figs. 4 and 5 represent acidophilic intranuclear inclusions at liver cells from *rhesus* injected with yellow fever virus (African strain).

Note in fig. 4 hypertrophy of nuclei presenting acidophilic granules and liver cells with "entamoeba-like" intranuclear bodies and in fig. 5 the remarkable amount of basichromatin condensed over the nuclear membrane. Round-shaped necrotic liver cell is evident in fig. 5.

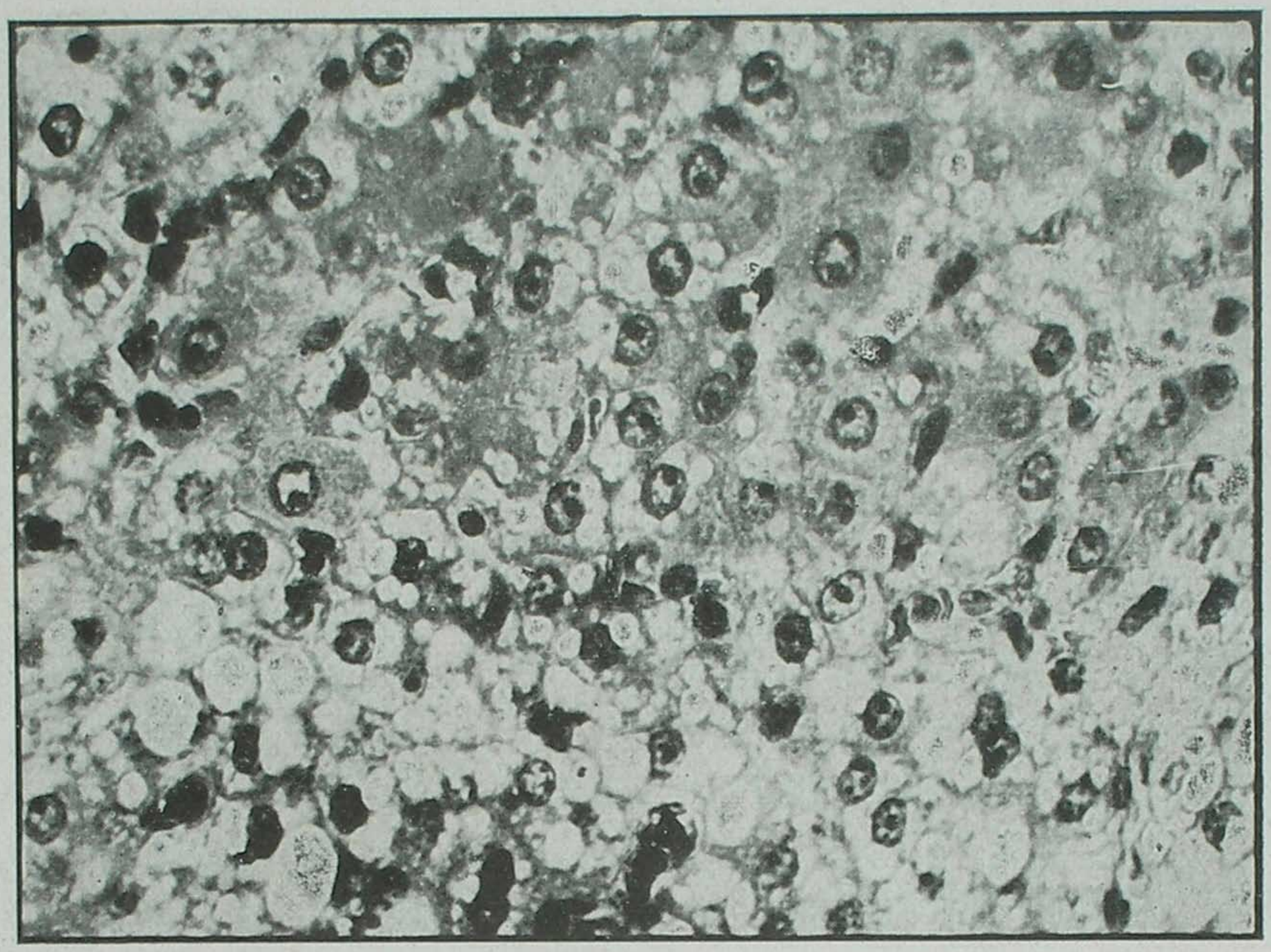

Fig. 1

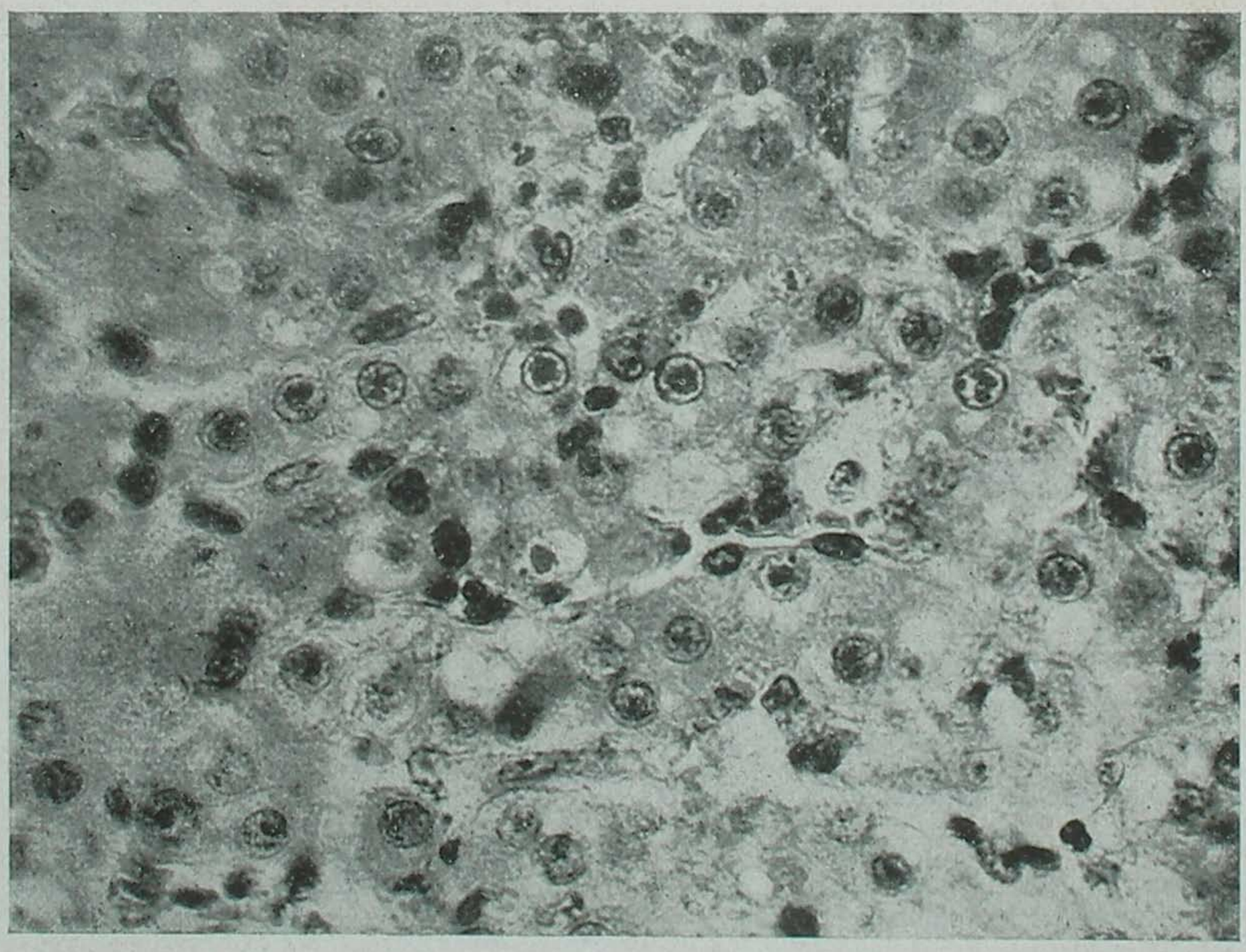

Fig. 2

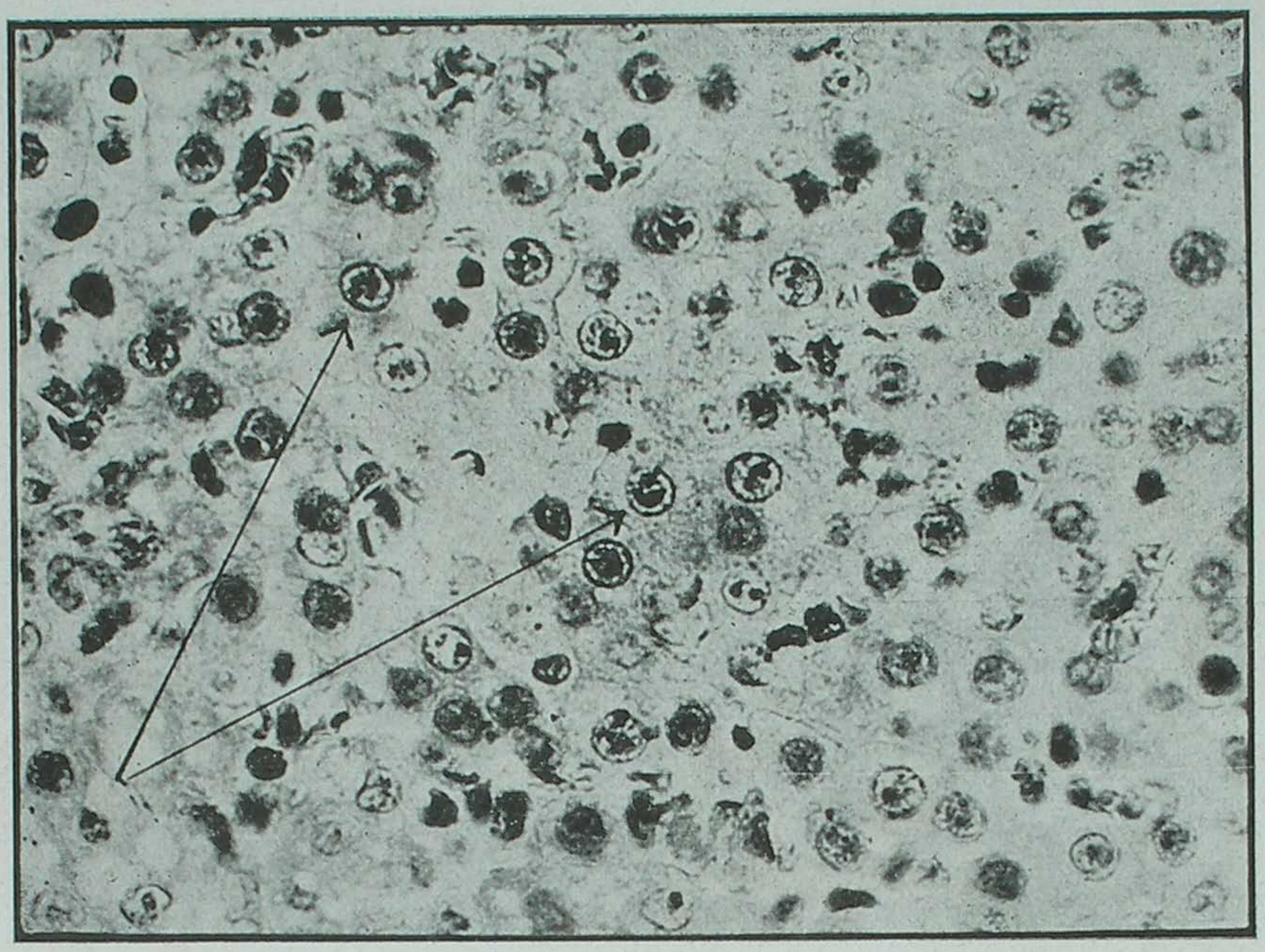

Fig. 3

Fig. 4

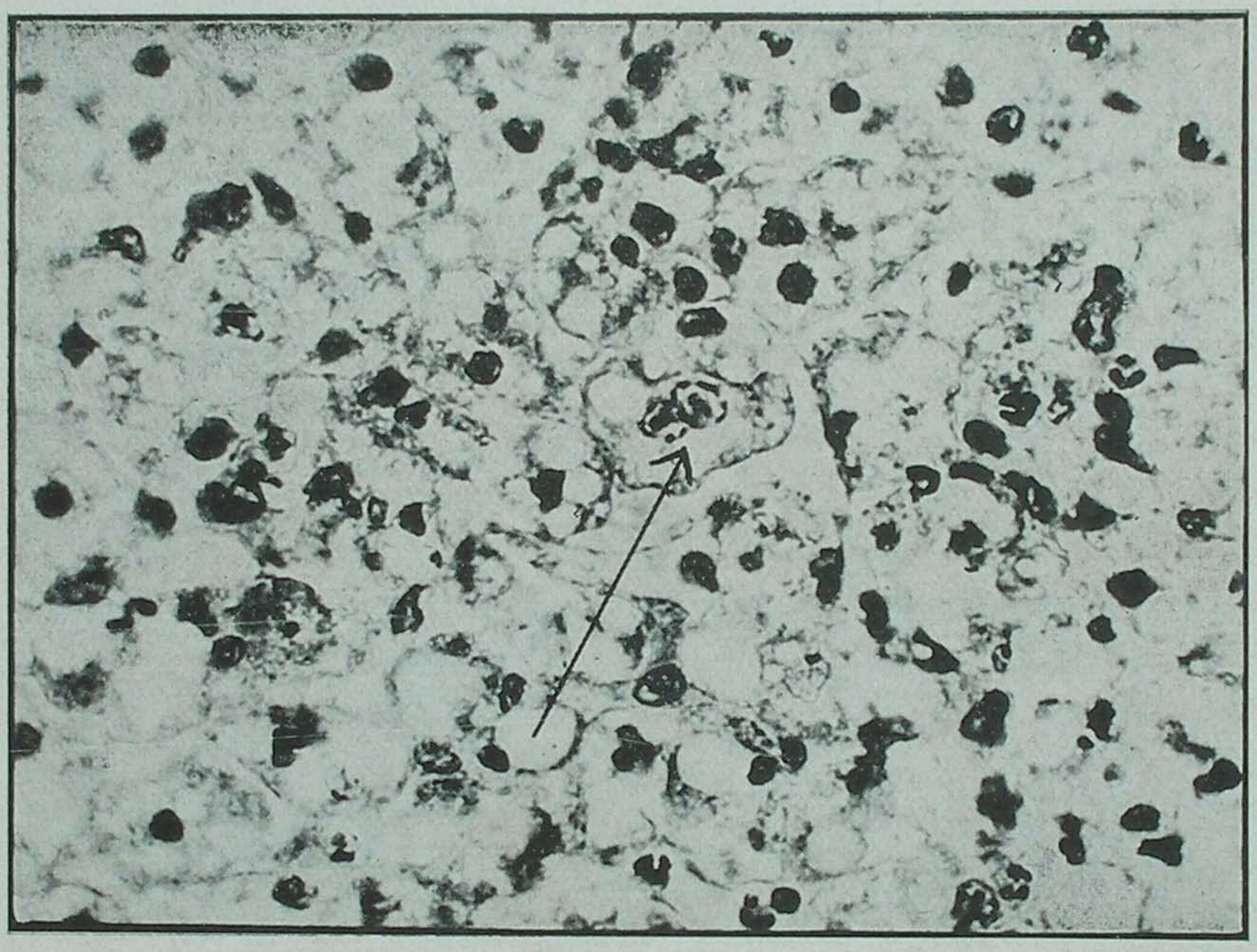

Fig. 5

sed of coagulated nucleoplasm, being surely of a different structure and composition from the two nuclear constituents, chromatin and nucleolus.

It is not definitely known whether the "inclusions" in virus diseases are purely degenerative in nature (*reaction products*) produced by the cell in response to injury or are composed in part at least of the specific virus itself. Nevertheless a desirable work, as emphasizes GOODPASTURE, will be that directed towards "recognizing a constant association of a characteristic type of inclusion with any particular infection, and by identifying it with the lesion so that a diagnosis of the infection on this basis may be possi-ble" (Amer. Jour. of Pathology, 1925, vol. 1, n. 1, pag. 2).

In fact, it was with this object in view that the pathology of experimental yellow fever have been considered in my late papers (5).

Finally, it has appeared that if acidophilic intranuclear inclusions (oxychromatic degeneration) occur both in monkeys infected with the Brazilian and in those with the African strains I was able to examine, discrepancies actually exist between the structure of the inclusion itself or the appearance of recognizable nuclear constituents in both series. They are as follows :

1. In liver cells from monkeys of the African strain series the acidophilic intranuclear material frequently presents itself subdivided in minute masses or granules as reported by STOKES, BAUER and HUDSON (Amer. Jour. of Trop. Med., 1928, vol. VIII, n. 2, pg. 157) and by HUDSON (Amer. Jour. of Pathology, 1928, vol. IV, n. 5, pg. 409); that "granular stage" (evidently an early one) is not so abundant in liver sections from monkeys of the Brazilian strain series, and above all the fragmentation of the acidophilic intranuclear material never is so marked, the granules being there less numerous and generally larger than in the African strain series.

2. In liver cells from monkeys of the Brazilian strain series the "butterfly-like" intranuclear figure (fig. *h*) is a common aspect in early stages of oxychromatic degeneration; such figures are surely not abundant in liver sections from the animals of the African strain series I could examine, and in fact I was unable to demonstrate in such sections "butterfly-like" bodies exactly similar to those recorded in monkeys injected with the Brazilian strain.

3. The amount of basichromatin that becomes marginated forming thick rods over the nuclear membrane at liver cells with intranuclear bodies is apparently more considerable in *rhesus* from the African strain series (predominance of later stages of oxychromatic degeneration?) than in those from the Brazilian strains series (predominance of early stages of oxychromatic degeneration?).

It is believed that differences in the virulence and adaptation of the two strains to *Macacus rhesus* expressed on the other hand by slight necrosis of liver cells at one series (Brazilian strains) and marked necrosis at another (African strain) may account for such discrepancies and surely further data are needed before definite conclusions may be stated as far as differences at nuclear changes in both series are concerned.

---

(5) Brasil-Medico, 1928, n. 36, pg. 1001, Sciencia Medica, 1928, n. 9, pg. 427. Mem. Inst. Osw. Cruz, Supl. n. 2, pg. 55, C. R. Soc. Biol, 1928, T. XCIX, pgs. 1655, 1660, 1669 and 1671.