A comparison of the inclusion bodies of alastrim and vaccinia in the monkey (Macacus rhesus) (*)

by

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(Plates XXVIII—XXXI)

Alastrim, called also amaas, Kaffir-milkpox, whitepox, mild smallpox, paravariola, parasmallpox, variola minor, variola mitigata is a specific contagious eruptive fever resembling smallpox, a mitigated form of that disease in the opinion of some (LESCHKE, McSWEENEY), a quite different disease closely related to it in that of others (GARROW).

Not many observations have been reported concerning the inclusion bodies of alastrim. In fact their mere existence is a matter of dispute (see RICARDO JORGE).

While a successful inoculation of the disease to the monkey is stated by several authors and different species 1 were tried, no microscopic examination of the experimental lesions has been published previous to our own reports in 1933.

If it should be shown that similarities or differences are found between the Guarnieri bodies of variola and the inclusion bodies of alastrim important data would be supplied concerning the identity of variola and alastrim. This is a fascinating line of work, as inclusion bodies correspond to rather delicate and highly specific cellular changes.

Considerable variation in their structure, however, do occur specially when different animals and tissues are compared. Yellow fever intranuclear inclusions, for instance, as seen in the brains of encephalitic monkeys inoculated with the mouse strain of yellow fever virus are sometimes similar, but more often different from typical intranuclear inclusions as they appear in liver cells (GOODPASTURE).

The object of this paper is to study by comparison the experimental infection of rhesus monkeys with the viruses of alastrim and

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1 "Rhesus and bonnet monkeys" (Green 1915); M. rhesus (Leake and Force 1923); M. rhesus (Blaxall 1923); "un Atele, Koata ou Koaita du Brésil" (Baujean 1925); "diverses espèces et variétés de cercopithèques" (Van_Hoof 1925); "Macacus cynomolgus" (Leake and Force 1927).
of vaccina. A great stress was put into the morphology of the inclusion bodies as they appear in epidermal cells of the experimental vesicles and pustules. Cytoplasmic inclusion bodies of alastrim were examined in specimens from 12 experimentally infected rhesus monkeys and vaccine bodies in specimens from three other rhesus monkeys. In the latter vesicles and pustules were induced after intravenous inoculation of recently prepared bovine vaccine emulsion. Alastrim material was derived from 3 patients during a small outbreak of the disease in 1932 at Rio de Janeiro City, from 4 patients during an outbreak in 1933 at Estado de Minas Gerais, and from one patient during another outbreak at Estado do Rio de Janeiro in 1933.

The requirements for a comparison between inclusion bodies were apparently fulfilled as they were examined in the same animal and tissue, and as the specimens were submitted to the same artefacts: fixation in Helly's fluid, embedding in paraffin, and staining with alum hematoxylin and eosin.

This report is based upon material from the following monkeys:

**Rhesus — 1469.** The content of one vesicle and of two pustules from J. P. S., a patient of alastrim (Hospital Oswaldo Cruz, Rio de Janeiro City) on the eighth day of disease was sucked up by means of three sterile capillary pipettes, and drawn up before driving into a tube containing approximately 1.5 cubic centimeter of sterile physiologic sodium chloride solution. The turbid suspension was injected intravenously to rhesus 1469 a few minutes latter no scarification being performed. The monkey presents a rise of temperature after five days and an eruption after six days. The first sign of the eruption was in the form of small separated vesicles upon the forehead, lower extremities, trunk and abdomen. These vesicles soon become pustular, the stage of papule being rather difficult to recognise in the monkey. During the next day or two more vesicles appear. Pieces of skin were excised from the trunk (n.º 3602) on the seventh day, and from the forearm (n.º 3618) and hip (n.º 3619) on the ninth. The general condition remained always good and the animal recovered.

**Rhesus — 1474.** The content of several pustules from rhesus 1469 on the seventh day of inoculation was collected in capillary pipettes and added to about 1 c. cm. of salt solution. The turbid emulsion was injected a few minutes latter to rhesus 1474 no scarification being performed. The animal developed fever (41.0 C.) on the fifth day after inoculation and an eruption on the eight day. Quite large sometimes hemorrhagic vesicles without any definite umbilication appear over the palms of the hands, soles of the feet and legs. Pictures from such skin lesions were given in a previous report (see C. R. Soc. Biol., 1933, pg. 917). Several pieces of skin were excised on the tenth and on the twelfth days from the internal aspect of the left leg (n.º 3632), right foot (n.º 3638), and right forearm (n.º 3639). The animal finally recovered and remained well.

**Rhesus — 1484.** Injected intravenously as described in the previous experiment with the content of several pustules from the above mentioned animal (rhesus 1474) on the twelfth day of inoculation developed a temperature of 39.5º C. on the fifth day after inoculation and an eruption on the seventh, more profuse upon the extremities rather than the face and the trunk. New vesicles which soon become pustular appear during the next three days. Several pieces of skin were excised on the eight and on the ninth day from the left hip (n.º 3663), left leg near the ankle, and left and right feet (n.ºs 3664 and 3665). The animal recovered and remained well.
**Rhesus — 1465.** The content of several pustules from a patient of alastrim (P. L., Hospital S. Francisco de Assis, Rio de Janeiro City) was withdrawn with several fine pipettes and a suspension in saline solution prepared. A few hours latter this material was transferred to the interorbital region of rhesus 1465 while many scarifications were made by means of a scalpel. Reddening and edema of the region appear four days latter, two small vesicles being then apparent over the scarifications and excised for microscopic examination (n.º 3993). The animal developed a temperature of 39.5º C. on the sixth day after inoculation and finally recovered and remained well.

**Rhesus — 1492.** A saline suspension prepared with the content of several vesicles and pustules from J. J. J., a patient of alastrim on the ninth day of disease (Hospital Oswaldo Cruz, Rio de Janeiro City) was injected intravenously to rhesus 1492. The animal developed a temperature of 39.6º C. on the sixth day after inoculation and an eruption appears in the form of a small vesicle over the external ear and a few more over the trunk but it remains quite discrete. The microscopic examination from a specimen excised on the ninth day shows the structure of a pustule in dessication. No inclusion bodies were detected and the material was discarded.

**Rhesus — 1577.** Injected intravenously on July 13, 1933 with an emulsion in saline solution of pustule contents from a patient of alastrim secured on July 9, 1933 by Doctor Jairo Lobo Martins at Caratinga, Estado de Minas Gerais, developed fever and a vesicular eruption on the sixth day after inoculation. Two vesicles from the leg were excised for microscopic study (n.ºs 3904 and 3905). The animal recovered and remained well.

**Rhesus — 1593.** Inoculated intravenously on August 9, 1933 with 2 c.c.m. of an emulsion in saline solution of pustule contents from a patient of alastrim secured on August 3, 1933 by Doctor Jairo Lobo Martins at Caratinga, Estado de Minas Gerais, developed a temperature of 30.º C. on the fifth day after inoculation and a vesicular eruption predominant upon the lower extremities on the sixth day. The animal did not succum.

**Rhesus — 1597.** Was injected intravenously on September 12, 1933 with 2 c.c.m. of an emulsion in physiologic salt solution of pustule contents mixed with glycerine secured on September 1, 1933 at Estado de Minas Gerais from G. P., a patient of alastrim. The material was obtained through the courtesy of Doctor Ermani Agricola, Director de Saúde Publica Estadual. At the same time the skin was scraped upon the back. The animal developed a temperature of 39.8º C. on the fifth day and reached 40.1º C. on the sixth day. On the fourth day several subcutaneous papules but no vesicles were seen at the area in which the skin have been previously scraped. On the sixth day numerous vesicles appear upon the lower extremities, and quite a few upon the forehead. Several vesicles were excised for microscopic examination, three (n.ºs 3983-85) on the sixth and others (n.º 3998) on the eight day after inoculation. On the seventieth day dessication was advanced producing crusts which loosen readily. The animal finally recovered and remained well.

**Rhesus — 1605.** Subinoculated intravenously from the above mentioned animal (rhesus 1597) developed a temperature of 39.8º C. on the fifth day. This continued for three days, and on the sixth several pustules and vesicles appear upon the lower extremities, and quite a few upon the forehead and trunk. On the seventh day new vesicles appear upon the legs and feet. The vesicles and pustules are numerous upon the forearms and better appreciated after the hairs were cut short. Two biopsies (n.ºs 4017 and 4018) were performed. The animal did not succum. The material was derived from two vesicles recently excised from rhesus 1597 on the seventh day after inoculation and soaked in physiologic salt solution.

**Rhesus — 1615.** Subinoculated intravenously from the above mentioned animal (rhesus 1605) developed a temperature of 39.8º C. on the fifth day. The temperature falls to 38.5º C. on the ninth
day and the animal recovered. The rash appears on the sixth day, and the lesions are more numerous upon the lower extremities as usual. New vesicles appear on the seventh and on the eight days. Three vesicles were excised for microscopical study (n.ºs 4035, 4051 and 4052). The material was derived from several pustules recently excised from rhesus 1695 on the eighth day after inoculation and soaked in physiologic salt solution.

Rhesus — 1692. Was injected intravenously on September 12, 1933 with 3 c. cm. of an emulsion in physiologic salt solution of pustule contents mixed with glycerine and also dried in capillary pipettes secured on September 1, 1933 at Estado de Minas Gerais from J. M., a patient of alastrim. The material was available through the courtesy of Doctor Ernani Agricola. On the fourth day developed a temperature of 39.8º C. and reached 40.4º C. on the seventh, falling to 38.7º C. on the ninth and remaining around 38.3º C. on the following days. The animal did not succumb. The rash appears on the sixth day, numerous vesicles being found on the seventh specially upon the lower extremities. On the eighth day numerous vesicles were seen upon the forearms, and quite a few upon the hands. On the twelfth day desiccation was advanced producing crusts. On the eighth day after inoculation two vesicles upon the hip were excised for microscopic study (n.ºs 3994 and 3995).

Rhesus — 4060. Injected intravenously with an emulsion in saline solution of pustule contents from L., a patient of alastrim on the tenth day of disease, developed fever on the sixth day after inoculation. This continued for three days and a vesicular eruption appears on the sixth day. A vesicle was excised for microscopic examination (n.º 4060). The animal recovered and remained well. The patient was brought to Hospital Oswaldo Cruz, Rio de Janeiro City, from Itaperuna, Estado do Rio de Janeiro, where an outbreak of alastrim was prevalent at the time.

Rhesus — 1629. Subinoculated intravenously from the above mentioned animal (rhesus 4060) developed a temperature of 40.1º C. on the fourth day. Fever continued for three days and on the fifth a few papules appear upon the lower extremities and forehead. On the sixth day numerous vesicles are seen upon the forehead, external ear and lower extremities. New ones appear on the seventh day. Two biopsies were performed, one (n.º 4076) on the eighth and another (n.º 4079) on the tenth day after inoculation. The material was derived from six small pustules recently excised from rhesus 4060 on the eight day after inoculation and soaked in physiologic salt solution. The animal recovered.

Rhesus — 1556. Was injected intravenously with standard bovine vaccine pulp recently prepared and developed a temperature of 39.4º C. on the fourth day after inoculation. This continued for two days and reached 40º C. on the sixth day. Several papules and vesicles which latter become pustular appear on the fifth day and new ones on the eight day after inoculation. Some of them ulcerate. Specimens from the skin were excised on the sixth day after inoculation (n.º 3791-94). The general condition of the animal becomes progressively worse and it was found moribund on the twelfth day after inoculation. A few drops of chloroform were administered and the necropsy was performed immediately.

Rhesus — 1559. Was subinoculated intravenously with the content of several pustules from rhesus 1556. The temperature rises five days after the inoculation when an eruption appear over the trunk and lower extremities. Several vesicles and pustules were excised on the fifth day (n.º 3802). The animal appears very ill and prostrated and died twelve days after inoculation.

Rhesus — 1571: Received intravenously 5 c. cm. of a suspension in physiologic salt solution of vaccinia pulp. The material was the same injected into rhesus 1556 as previously reported but it was placed in cold storage during 85 days. The monkey developed fever and an eruption appear on the sixth and seventh day of inoculation. New vesicles appear on the sixth and seventh days when specimens from the skin were excised for examination (n.ºs 3855-3860). The animal showed signs of illness for thirteen days, but it finally recovered and remained well.
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Rhesus—1970. Was injected intravenously with the content of five vaccine emulsion capillary tubes ready for the market. The animal showed no reaction and remained well.

CYTOLOGICAL CHANGES IN ALASTRIM

The cytological appearances investigated comprise chiefly cytoplasmic changes as they appear in cells from the *stratum germinativum* of the epidermis. They resemble in a general way the Guarnieri bodies of variola and vaccinia.

Nuclear changes while present (Fig. 22) were rather scanty and will be discussed latter.

In some cases, while no inclusion bodies are seen, there may be basophilic material attached to the nuclear membrane (Figs. 4 and 10) and vacuolation of the surrounding cytoplasm. In other cases, while no definite inclusion body is yet visible, the cytoplasm of the epidermal cell assumes in a limited extent a blue stain with hematoxylin; the boundaries between such basophilic and normal acidophilic cytoplasm are not clear and the reticular structure remarkably well preserved in the basophilic areas (Figs. 5, 6 and 33).

Alastrim bodies as they appear after fixation in Helly's fluid and staining with hematoxylin and eosin are illustrated in Plates XXXVIII and XXIX, Figs. 1-23. They may be seen as irregular deep blue to bluish-violet masses which occasionally indent the nuclei (Figs. 1 and 2). Sometimes the deeply stained bodies surround the nuclei in a peculiar way resembling a small cap or coil (Figs. 7, 8 and 34) although not indenting the nucleus. They do not fuse likewise with the nuclear membrane (Fig. 34).

Alastrim bodies are very polymorphic, triangular (Figs. 19 and 37), crescent-shaped (Figs. 9, 20 and 38) or racket-like (Fig. 17). Most of them are scattered in the cytoplasm (Figs. 1 and 11), sometimes near two opposite ends of the nucleus (Fig. 14). As a rule they are single when rather voluminous (Figs. 35-40). Such advanced stages are associated with larger areas of unstained cytoplasm (Figs. 36, 39 and 40).

The basophilic reaction in sections colored by hematoxylin and eosin can be seen even in such alastrim bodies which are as large as the nucleus (Fig. 9). In one case represented in Figure 16, one basophilic and one acidophilic body were found side by side in the same cell. Such appearances were, however, very rare.

Finally, Figure 21 illustrates a necrotic cell of the *rete Malpighi* in which the cytoplasm is deeply stained by the eosin and the nucleus is pyknotic. The inclusion body appears as a pinkish violet rounded mass in an apparently structureless cytoplasm. It is open to question if the
marked acidophilic reaction of the alastrim body is not here dependent upon non-specific necrotic changes.

From the foregoing description it is evident that the chief characteristics of the alastrim bodies which appear in cells of the stratum mucosum of Silenus rhesus are their basophilic reaction and their occurrence as a single body in latter stages.

In some cases the nuclei of cells provided with alastrim inclusions present a normal structure (Figs. 3, 13, 34, 35, 36 and 37). In other cases they are swollen (Figs. 9, 10, 12 and 17) or shrunken (Figs. 16, 19, 20 and 35). Intranuclear inclusions as they are illustrated by COUNCILMANN, MAGRATH and BRINCKERHOFF in their Plate IX, Figures 5 7, 8 and 9 were not found. In a few cells, however, the nuclear changes could not be properly described as pycnosis, karyorrhexis or karyolysis. Figure 22 shows one of those cells in which acidophilic material appears in the nucleoplasm and is enclosed by marginated basophilic chromatin, an appearance which suggests to some extent oxychromatic degeneration.

Advanced stages of cytoplasmic inclusions reveal a striking similarity although several strains of the virus of alastrim were at work. No individual variation was equally noticed as it may be seen when Figures 10-16 from rhesus 1465 injected with strain P. L. are compared with Figures 4-9 from rhesus 1469 and Figures 17-23 from rhesus 1474 both injected with strain J. P. S. Those strains were derived from cases during an outbreak of alastrim in 1932 at Rio de Janeiro City. Alastrim bodies indistinguishable from those above refered to were also found in rhesus 1579 and rhesus 1605, strain G. P. and in rhesus 1602, strain J. M., both strains derived from patients during another outbreak of the disease in a different place (outbreak of 1933 at Estado de Minas Gerais) as well as in rhesus 1577 and 1593, strain from Caratinga, and rhesus 4060 and 1629, strain from Itaperuna, Estado do Rio de Janeiro.

As one would expect, however, while several stages were usually found in every block some of them appeared quite numerous. This depends obviously from the stage of the lesion. Small alastrim bodies for example, were easily found in rhesus 1484 (Figs. 1, 2 and 3) while advanced stages (Figs. 19, 20 and 21) were quite common in rhesus 1474 and cap-shaped bodies (Figs. 7 and 8) in rhesus 1469; all the animals were injected, however, with the same strain of the virus (strain J. P. S.).

As a rule, alastrim bodies are more numerous in early lesions of the skin, namely in the stage of epidermic papule. They become less
and less numerous while suppuration advances. Large alastrim bodies, however, are not rare in epidermal cells from the walls of a pustule. They could no more be demonstrated in a desiccated pustule.

No attempt is made in this paper to examine the changes produced in mitochondria an in the Golgi apparatus by the virus of alastrim. Alum hematoxylin and eosin stained sections have been made the basis of the work. This stain so used for general histological work presents no difficult point and the results are very regular. Biondi-Heidenhain and Giemsa’s methods should be more desirable. But the proper combination in Biondi-Heidenhain staining solution and the differentiation step in Giemsa’s method certainly increase the difficulty in obtaining perfectly comparable stained preparations.

Definite heterogeneity of the alastrim bodies may be evidenced even in hematoxylin and eosin stained preparations. Some of them show very small spherical areas (Figs. 9, 14, 15 and 38) which do not take any stain but appear clear resembling unstained vacuoles. Other inclusions show a definite reticular structure (Fig. 20) and seem to grade into the cytoplasm (Figs. 14, 18, 37 and 38), probably a false impression due to the methods of fixation and staining.

In fixed preparations clear-looking structureless spaces are very conspicuous in the cytoplasm of epidermal cells provided with inclusions (Fig. 21) when sunlight is used and the sections are examined with a 2 mm. oil-immersion objective, Zeiss compensating ocular 6, the iris diaphragm and the Abbé condenser properly focussed so as best to illuminate the preparation. Nevertheless, when optical conditions are changed, a feltwork will come to light in these clear spaces. Figure 23 shows the cell represented in Figure 21 as seen when an arc lamp and a 2 mm. immersion objective and Leitz ocular 25 are used. The whole net resembles the one existing in the cytoplasm of normal epidermal cells except for their lack of staining with eosin. It appears sometimes poorly stained with hematoxylin. Figures 39 and 40 represent also the same cell reproduced in Figures 21 and 23. In Figure 40 the light was partially shut off by the iris diaphragm and a network can be distinguished in parts of this cell corresponding to clear-looking spaces more readily than in Figure 39. A framework similar in appearance was found also in the vacuolated cytoplasm near alastrim bodies in the cells represented in Figures 13, 15 and 19.

In some cells provided with inclusions an acidophilic filament or membrane is a very conspicuous structure demarcating the clear-looking spaces in the cytoplasm (Figs. 9, 16, 19, 21, 38 and 40).
CYTOLOGICAL CHANGES IN VACCINIA

Vaccine bodies of about the same size as the nucleolus are represented in Figures 24, 25, 41 and 42. Circumnuclear in some cells (Figs. 24 and 25), they may spread throughout the cytoplasm in others (Figs. 41 and 42), being either single (Fig. 24) or multiple and in some cases related to opposite ends of the nucleus (Figs. 25 and 42).

Their staining is not consistent in hematoxylin and eosin stained preparations. Some of them (Figs. 25, 26 and 28) are tinged in grayish-violet to deep blue (basophilic reaction), others (Figs. 24 and 27) a purplish-red to pink (acidophilic reaction).

Small clear spaces or unstained vacuoles may be seen in some of the vaccine inclusions (Fig. 28), while in others a reticular structure is apparent (Fig. 42).

Irregular vaccine bodies diffusely distributed through the cytoplasm are illustrated in Figures 29, 30, 31, 32, 43 and 44. Some of them reach a relatively large size. They occur in irregular clumps and masses scattered through the cytoplasm and sometimes in fine and coarse granules. When numerous they lie in a clear area often well demarcated from the adjoining cytoplasm by a wavy pink stained filament or membrane (Figs. 31, 32 and 41).

Vaccine bodies do not stain alike within the same cell. While some of them are stained a grayish-violet, others present a definite pink color. This variation in their staining within one cell was a prominent feature of the specific cellular inclusions of vaccinia in epidermal cells of the rhesus monkey.

The structure of the nucleus may be normal in epithelial cells bearing numerous vaccine bodies (Fig. 29). In most cases, however, the nuclei are swollen (Figs. 25, 26 and 27) or shrunken (Figs. 30, 31 and 32).

DISCUSSION

Describing several appearances in cytoplasmic changes in alastrim and vaccinia it may not be inferred that they necessarily correspond to gradual transitions of those structures. Naturally this is the case with some of them. But it must be asked whether similar appearances should not correspond to different degrees in which the injured cells react to the virus. In this case and notwithstanding their occasional relative small size they really may correspond to a final stage of specific changes being the only effect which can be induced by the virus in the cell.
The first impression one gets from a study of specific cellular inclusions in alastrim and vaccinia as they appear in skin lesions of rhesus monkeys is their extreme variation obviously dependent on intermediate stages in the formation of inclusions or related to different degrees of cell injury in response to the virus.

However, when small and discrete, they look very similar in both diseases as may be seen if Figure 11 representing two small alastrim bodies at the opposite ends of the nucleus is compared to vaccine bodies illustrated in Figure 25.

Nevertheless, on closer examination, definite although slight and delicate differences may be noticed between alastrim and vaccine bodies. They are chiefly related to the number of inclusion bodies within the cell and to their staining.

Astrastrim bodies are usually single (Figs. 2, 3, 8, 9, 12, 13, 15, 17, 18, 19 and 20) even in such advanced stages in which they have the approximate size or are even larger than the nucleus of the epithelial cell (Figs. 9 and 20). In early stages, however, two and sometimes three or four small paranuclear or circumnuclear bodies may be found (Figs. 1, 7 and 11).

If there is a resemblance between early stages of alastrim and vaccine bodies, as previously stated, a striking difference is to be noted when advanced stages are compared. In fact, while alastrim bodies in late stages occur singly, vaccine bodies are regularly numerous within the epithelial cells of monkeys (Figs. 29-32) in advanced stages leading to extensive vacuolation of the cytoplasm. It seems that this appearance is not unusual with advanced stages of vaccine bodies whatever may be the tissue or the animal examined because illustrations showing numerous large irregular vaccine bodies within epithelial cells of ectoderm of the chick embryo are given by GOODPASTURE, WOODRUFF and BUD-DINGH in their Figure 1, Plate 42, and within corneal cells of the rabbit by HUEKEL in his Figures 93 and 113, Plate 111.

The other differences regards the staining reactions of the inclusion bodies.

Astrastrim bodies do stain regularly a deep blue when they present a small size (Figs. 1, 7, 8 and 12) to grayish-blue when about the same size of the nucleus (Figs. 9 and 20), whereas vaccine bodies present a very capricious staining. Even when they are small and about the size of the nucleolus, vaccine bodies sometimes are definitely pink stained by eosin (Fig. 24), while in most cases their staining is a blue one (Figs. 25, 26 and 28).

The most characteristic feature of vaccine bodies, however, is
the occurrence within the same cell of inclusion bodies stained blue while others show a definite pink staining (Figs. 29-32).

After noticing how numerous were such polychromatophilic inclusion bodies in vaccinia lesions of the monkey, we tried a systematic search for them in alastrim. As a result, the epithelial cell represented in Figure 16 was found in which two crescent-shaped alastrim inclusions are seen, one staining blue, the other pink. Of course this was the only instance in which polychromatophilic alastrim bodies could be demonstrated in the monkey. This is in strong contrast with the marked polychromatophilia remarked in latter stages of vaccine bodies.

SUMMARY

1. — Vesicles and pustules containing numerous cytoplasmic inclusion bodies within the epidermal cells were regularly produced in monkeys (Macacus rhesus) by intravenous inoculation either of alastrim virus or of recently prepared vaccine emulsion, no previous scarifications being required. Alastrim virus seems less virulent for this species of monkey than the virus of vaccinia is. While 12 rhesus monkeys injected intravenously with seven strains of alastrim virus developed regularly an experimental infection and finally recovered and remained well, the intravenous injection of vaccine virus was followed by death in two from four rhesus monkeys injected.

2. — Slight but definite and regular differences were noticed between alastrim and vaccine bodies after fixation in Helly’s fluid, embedding in paraffin and staining with alum hematoxylin and eosin. They are related to the number of inclusions within the cell and to their staining.

3. — In each cell of the epidermis of Macacus rhesus there is usually a single alastrim body when late stages are considered, and quite a few (2-4) when early stages are observed. Alastrim bodies stain a deep blue to grayish-blue. Nevertheless, in necrotic epidermal cells or in cells provided with a few small inclusions they sometimes present a pink staining.

4. — Vaccine bodies in late stages of development are numerous in each epidermal cell of the rhesus monkey and present regularly a characteristic polychromatophilia.

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