3419

Considerations on the Relationship of the Reticulo-Endothelial System to Kala-Azar.

C. H. HU AND J. R. CASH.

From the Department of Pathology of the Peking Union Medical College.

These experiments attempted to analyze more closely the nature of the characteristic cellular reaction occurring in the tissues in kala-azar. The hamster *Cricetulus griseus,* a variety of Chinese field mouse, which has been shown to be highly satisfactory for this purpose, was used.[1]

Our first observations were upon the blood obtained by splenic puncture from both normal and infected animals. Differential counts upon preparations supravitally stained with neutral red and janus green by Sabin's technique were made from 20 normals, and 20 infected experimentally with kala-azar, from 10 to 12 weeks.[2] At this time the infection is always well established and the lesions extensive.

The results of these counts are given in the following table.

TABLE I.
Differential Counts Upon Supravitally Stained Splenic Blood.

Type of cells.	Average count of 20 normal Hamsters.*	Average count of 20 Hamsters infected with Kala-azar.*
	Per cent	Per cent
P. M. N.	2.59	2.87
P. M. E.	0.93	0.41
P. M. B.	0.02	0.02
Monocytes	1.70	1.84
Reticulo-endothelium (clasmatocytes)	1.07	6.21
Lymphocytes	91.74	78.93
Unidentified	1.95	9.72

*No. cells counted: 1000 to each animal.

By this method one has little difficulty in identifying practically all of the cells seen in preparations made from normal animals. The great increase, in unidentified cells in infected animals, is caused by the increase in number of definite type of small, mononuclear cell which is very actively motile, and which never has been observed to contain kala-azar bodies. As yet we are uncertain as to the exact identity of this cell, but it clearly seems to have nothing directly to do with the propagation or destruction of the Leishmann-Donovan

bodies, and in the tissues, forms no specific part of the lesions. The distinction between monocytes and reticulo-endothelial cells of clasmatocytes was based upon their characteristic receptive staining with neutral red, the monocyte showing a rosette of the dye opposite the "Hof" of the nucleus, while the clasmatocytes of the splenic reticulo-endothelial system take up the stain in large, irregular globules.

Leishmann-Donovan bodies, which stain slightly with neutral red, were found in practically all of the reticulo-endothelial cells of the infected animals, but were never noted in the cells having the characteristic morphology of monocytes. Such supravital preparations are generally swarming with extracellular parasites, which have been liberated by the trauma of the splenic puncture. By a few moments careful observation, one sees the neutrophilic leucocytes quickly phagocytize many of these free kala-azar bodies. Films made from the splenic blood and stained with Wright's stain show that practically all of this phagocytosis takes place upon the slide, for only occasionally are neutrophiles seen which contain parasites.

Many observations made upon the peripheral blood show but little difference between the normal and infected animals. Parasites were found in the latter in almost negligible numbers, and then only in neutrophilic leucocytes.

After these studies were completed each animal was immediately killed and an autopsy performed. Carefully prepared sections show that the parasites are always intracellular, as has been previously noted. For further description of the lesions the reader may refer to a recent paper by Meleney and the accompanying literature.[3]

Upon studying the sections of an animal infected with kala-azar, one is immediately struck with the similarity of the lesions, caused by this disease, to the changes produced in the tissues of experimental animals by repeated injections of India ink. The idea that we may be dealing with the same fundamental cellular reaction in both instances soon suggested itself.

A series of hamsters infected with kala-azar were given small injections of India ink intravenously, and the animals killed on the following day. Sections showed that lesions in the various organs were limited to the reticulo-endothelial system which had undergone tremendous hyperplasia. Practically all of the cells containing kala-azar parasites likewise contained granules of ink, and very few ink-containing cells, which did not also contain parasites, were seen. In another series, which first received India ink and was then infected with kala-azar, the same picture was seen after the infection was well established.

In studying the skin of these animals many large cells filled with Leishmann-Donovan bodies were seen in the subcutaneous tissue and in all layers of the skin as well. These cells, of course, cannot phagocytize ink-granules injected intravenously, but when a small amount of ink was injected subcutaneously they immediately engulfed great quantities of it, as did all of the other parasite-containing cells in the various organs. Supravital stains upon small bits of subcutaneous tissue spread out upon the slide showed these cells to be clasmatocytes. These cells are the wandering cells of the reticulo-endothelial system and possess many, if not all, of the properties of the Kupffer cells of the liver and reticulo-endothelium of the spleen, bone-marrow, lymph glands and other organs.

We should like to emphasize the wide extent of the lesions in the skin and subcutaneous tissues of the hamster in kala-azar, which we feel has not been sufficiently recognized. The following illustrations are good examples of sections of skin taken at random from any heavily infected case. Clasmatocytes loaded with parasites in the skin and subcutaneous tissue thus cover the animal with a thick layer of infected cells. It is, therefore, surprising that so many attempts to transmit kala-azar from hamster to hamster by various biting insects have failed.

Summary. Supravital staining of the blood obtained by splenic

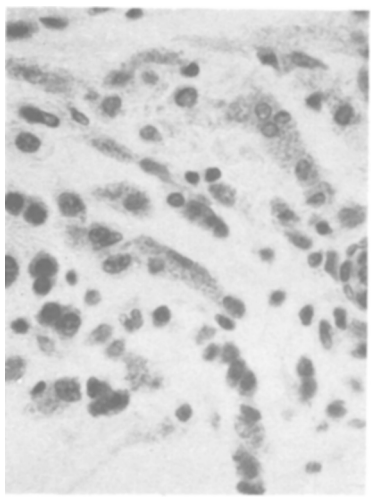

FIG. 1.
Section of skin of hamster infected with Kala-Azar. Several clasmatocytes containing Leishmann-Donovan bodies can be seen in the cutis. Hematoxylin and eosin x 680.

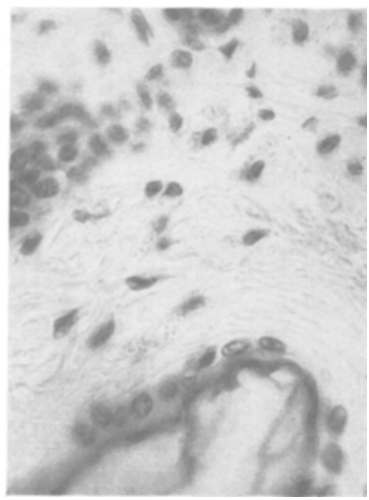

FIG. 2.
Section of subcutaneous tissue of the same animal showing many clasmatocytes loaded with Leishmann-Donovan bodies. Hematoxylin and eosin x 680.

puncture of hamsters infected with kala-azar shows that the large phagocytic cells, which form the characteristic lesions of the disease and contain the kala-azar parasites, have the staining qualities of reticulo-endothelium.

By marking out the cells of the reticulo-endothelial system of animals infected with kala-azar with intravenous and subcutaneous injections of India ink, one sees that the distribution of the lesions and of the parasites is practically limited to this system.

Attention has been called to the nature and wide extent of the lesions in the skin and subcutaneous tissue.

This is a preliminary report.

---

[1] Young, C. W., Smyly, H. J., Brown, C., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 357-359.

[2] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277.

[3] Meleney, H. E., *Am. J. Path.*, 1925, i, 147-168.

3420

Antigenic Character of Denatured Egg Albumin.

HSIEN WU, CARL TEN BROECK AND CHIEN-PIEN LI.

From the Departments of Biochemistry and Pathology, Peking Union Medical College.

The object of the present study is to determine the effect of denaturation on the immunological properties of egg albumin, and to show the relation between the albumins denatured by different methods.

The agents of denaturation used in our study are dilute acids and alkalies, alcohol and heat. Proteins denatured by these different agents have been variously called acid albumins, alkali albuminates, heat coagulated, and, alcohol coagulated proteins. Superficially these substances are similar, and previous work[1, 2, 3] from this laboratory has shown that they are indeed essentially the same in chemical and physical properties. This we have tried to verify by biological methods.

The acid and alkali albumins were prepared by mixing 1 per cent egg albumin solution with equal volumes of H/10 HCl and N/10 NaOH. After 2 or 3 days the denatured egg albumin was precipitated by neutralization and purified by repeated precipitation. The heat-denatured albumins were prepared by heating 100 cc. of 1 per cent egg albumin solution to which 1 cc. of N HCl or N Na_2CO_3