

Negative Findings of Leucolysin in the Sera of Cases of Kala-azar.

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Leucopenia is one of the outstanding features in cases of kala-azar. Its pathogenesis is still obscure. By incubating washed leucocytes of the blood of normal individuals with the sera of other normal individuals and of cases of kala-azar, Maggiore and Sindoni¹ found that there was a reduction in number of leucocytes with kala-azar serum, but no reduction with the normal serum. They, therefore, concluded that in the serum of kala-azar cases there was a leucolytic substance, "leucolysin," which is responsible for the occurrence of leucopenia. Their observation was later confirmed by other Italians,^{2,3} but Teng and Forkner⁴ showed that inoculation of killed *Leishmania donovani* into rabbits failed to produce leucopenia in these animals. In order to confirm or disprove the conclusion of the Italian workers we repeated their experiments and the results are briefly reported below.

Two cc. of blood was withdrawn from the arm vein of a normal individual. After a drop of blood from the tip of the needle was taken, immediately following its withdrawal from the vein, for the initial count of the white blood cells, the blood in the syringe was quickly forced into a 15 cc. centrifuge tube containing 12 cc. of citrated normal saline. The mixture was thoroughly stirred by a glass rod and centrifuged for 3 minutes at medium speed. The supernatant fluid was then removed, fresh citrated saline was added, the mixture again thoroughly stirred, and at the end of the third centrifugation the sediment was made up to its original volume of 2 cc. with citrated normal saline. One cc. of this blood cell suspension in saline was now put into each of 2 small tubes and another white blood cell count was made. After this 2 drops of kala-azar serum (obtained from etiologically proven but untreated case of kala-azar) were added to one tube and 2 drops of normal serum (obtained from another normal individual whose blood belongs to the same group as that of the kala-azar patient and the individual

¹ Maggiore, S., and Sindoni, M., *La Pediatria*, 1917, **25**, 81.

² Jemma, G., *La Pediatria*, 1920, **28**, 1081.

³ Arena, G., *La Pediatria*, 1927, **35**, 465.

⁴ Teng, C. T., and Forkner, C. E., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 661.

supplying the 2 cc. of blood) were added to the other. Another white count was then made. The tubes, now properly corked, were then incubated in a water bath for 2 or 3 hours and white counts were made at hourly intervals from each tube. The blood was thoroughly stirred with a glass rod before the sample was taken for each counting.

The above experiment was made with the serum of 6 cases of kala-azar. It was observed that in all these cases there was always a reduction in the number of leucocytes after several times of washing and after prolonged incubation with repeated stirring. The degree of reduction in the number of the leucocytes of the blood incubated with kala-azar serum was approximately the same as that with normal serum. The reduction was apparently due to the fact that many of the leucocytes in the suspension became attached to the stirring rods and the wall of the incubating tubes, as demonstrated by the microscopic examination of the stained blood films on their surfaces and further proved by the fact that with very thorough cleansing or paraffin coating of the glassware the degree of reduction became much less noticeable. It was, therefore, concluded that in the kala-azar serum there is no such substance as leucolysin. The lack of any leucolytic substance was further confirmed by the direct observation under microscope of the leucocytes of normal blood mixed with kala-azar serum. These leucocytes were found to remain either actively motile or at least physically intact even after 3 hours' incubation.

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Pigmented Cells in Adrenals and Testes of Hypophysectomized Rats.

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Brief mention has already been made of the fact that hypophysectomy in the rat is followed by the appearance of yellowish pigmented cells in the interstitial spaces of the testis and in the reticular zone of the adrenal.¹ The pigmented cells which we have seen in the testis seem to be similar to those described by Rasmussen² as occurring in

¹ Cutuly, E., McCullagh, D. R., and Cutuly, E. C., to appear in *Am. J. Physiol.*

² Rasmussen, A. T., *Am. J. Anat.*, 1917, **22**, 475.