

PRODUCTION OF CHARCOT-LEYDEN CRYSTALS FROM EOSINOPHILS WITH AEROSOL MA

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CHARCOT-LEYDEN crystals were first described in 1853 by Charcot,¹ and again in 1872 by Leyden.² They still remain enigmatic structures, and there are many conflicting reports in the literature as to their nature and significance. Their chemical nature is undetermined and there is considerable question as to whether they may be formed from normal blood. Thus, Liebreich³ stated they may be produced from every normal human blood. Also Neumann⁴ was able to produce them in normal blood. On the other hand, Thompson and Paddock⁵ in a study of the blood of 100 routine hospital admissions found no Charcot-Leyden crystals. There is also considerable question as to whether all eosinophils are capable of forming crystals and whether they are specific for eosinophils. Schwarz⁶ stated that the crystals are not an essential component of the eosinophil, since they could not be produced from the blood of all patients with eosinophilia. Again there are reports of the presence of crystals in the absence of eosinophils. Up to the present time there has been no method by which the crystals could be produced with certainty from eosinophils.

It is known that the crystals usually occur in association with eosinophils and that they are remarkably resistant to certain deleterious influences. Harrison⁷ has isolated the crystals in pure form from minced leukemic spleens containing the crystals. The crystals have been described in a diverse number of diseases: In the sputum of asthmatics, in leukemic blood and tissues, in allergic nasal polyps, in the blood and tissues of patients with periarteritis nodosa, in the feces in amebiasis, in the feces in helminthiasis, and in the bone marrow of sickle cell anemia. The crystals appear limited to primates; there is only one questionable case in which they were reported in the blood of a frog.

The purpose of this paper is to present a method by which Charcot-Leyden crystals may be produced rapidly and with certainty from eosinophils by means of Aerosol MA*, and to show that the crystals may be produced in the blood of a high percentage of normal persons and routine hospital admissions.

METHOD

Four and one-half cc. of blood obtained by venipuncture is mixed with 0.5 cc. of 3.8 per cent solution of sodium citrate. The blood is centrifuged and the buffy coat is removed by means of a capillary pipet. Two separate drops of this buffy coat are placed on a microslide. One of the drops is covered with a cover slip containing Aerosol MA and the other drop with a plain cover slip. According to Beeler,⁸ Aerosol MA is dihexyl sodium sulfosuccinate. It is a homologue of Aerosol AY, Aerosol IB, and Aerosol OT, and is a commercially pure, white, waxlike compound which is somewhat hygroscopic. The solu-

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*American Cyanamid Co.

bility is 34.3 grams per 100 cc. of water at 25 C. It has been used as a wetting agent, emulsifying agent, and detergent. Aerosol MA is rubbed over about half of the surface of the cover slip. The waxlike nature of the compound permits it to adhere readily to the surface. The cover slips are rimmed with petrolatum and the preparation is placed in a moist chamber to prevent drying. It is observed every twenty-four hours for seven days and the presence or absence of Charcot-Leyden crystals recorded.

The red cells contained in the buffy coat lyse immediately on application of the cover slip containing Aerosol MA, as do most of the leukocytes. The granules of the eosinophils and the Charcot-Leyden crystals do not lyse. The cells in the control preparations show little or no lysis for several days, and then as a result of bacterial growth. In the Aerosol MA preparations Charcot-Leyden crystals form within a few minutes to several hours, while in the control preparations an appreciable number of crystals do not form for seventy-two hours.

Permanent preparations may be made by removing the cover slip with a sliding motion, drying in air, fixing for one minute with absolute methyl alcohol, and staining with the usual hematoxylin-eosin technic. The eosin should be slightly acidified for brilliant coloration.

RESULTS

The blood of 100 routine hospital admissions was studied by this method and the results are shown graphically in figure 1. In the experimental group, in which the buffy coat was exposed to Aerosol MA, Charcot-Leyden crystals formed in 99 per cent of the cases by the first day. These all remained positive on the second, third and fourth days. On the fifth day one slide became negative for crystals to give a value of 98 per cent. Another slide became negative on the sixth and seventh days to give a final value of these last 2 days of 97 per cent. In the control group, none of the slides were positive the first day, 3 were positive the second day, 12 on the third day, 44 on the fourth day, 64 on the fifth day, and 75 on the sixth and seventh days. The figure 75, however, does not give a true value for the total number of cases that were positive, since on the latter two days as some slides became positive, others became negative. Actually, 80 per cent of the control group showed Charcot-Leyden crystals at one or more times.

In no case in the experimental group were Charcot-Leyden crystals found in the absence of eosinophils. In the control group, most of the cases which failed to show Charcot-Leyden crystals did show eosinophils.

It should be emphasized that the experimental preparations contained crystals in large numbers, directly proportionate to the number of eosinophils, and that the control preparations never showed crystals in the numbers seen in the corresponding experimental preparations.

The 100 hospital admissions were unselected and had a variety of diseases, such as hypertension, diabetes mellitus, Hodgkin's disease, abscess of the prostate, varicose veins, pneumonia, and fractures. Thirty-seven per cent of the patients had a total white count of over 10,000. In no patient was the total white count under 5,000. In 12 per cent of the patients there was an eosinophilia of over 5 per cent, the highest being 12 per cent. In 14 per cent of the cases the diagnosis was not determined. In the remaining 86, only 5 had the diagnosis of a possible allergic disease. There was no case of asthma, allergic rhinitis, or serum sickness.

The blood of 24 normal students was studied with the same technic, the results of which are shown in figure 2. In the Aerosol MA preparation 90 per cent were positive the first day for Charcot-Leyden crystals and remained positive throughout

the seven days of observation. In the control preparations, none of the slides were positive on the first and second days, 8.7 per cent were positive on the third day,

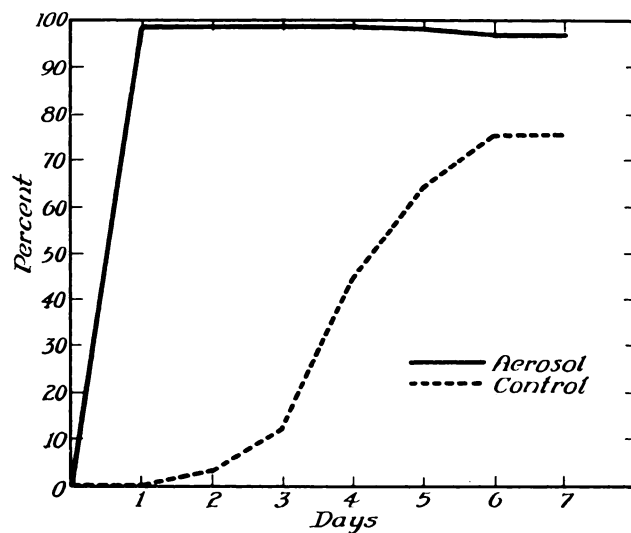


FIG. 1.—CHARCOT-LEYDEN CRYSTALS IN AEROSOL AND CONTROL PREPARATIONS IN 100 ROUTINE HOSPITAL ADMISSIONS.

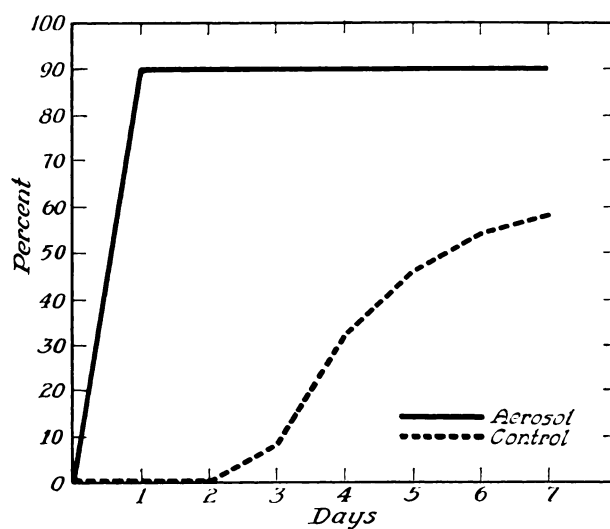


FIG. 2.—CHARCOT-LEYDEN CRYSTALS IN AEROSOL AND CONTROL PREPARATIONS IN 24 NORMAL PERSONS.

31 per cent on the fourth day, 46 per cent on the fifth day, 54 per cent on the sixth day, and 58 per cent on the seventh day. All the control and experimental slides remained positive throughout the seven days.

The shape of the Charcot-Leyden crystal is that of a hexagonal pyramid with bases opposed, as shown in figures 3 and 4. Their hexagonal shape in cross section is well illustrated in figure 5. This is a paraffin section of eosinophilic granuloma of bone, stained with Gram-Weigert's stain. Their size is ordinarily described as from 7 to 21 microns in length. In this work some crystals were just visible with the oil immersion lens, while others measured 96 microns in length and 9 microns in width. The crystals stain black with iron-hematoxylin and red with hematoxylin-eosin. In fresh preparations they are colorless or have a light yellow tint.

Many observations were made to determine the origin of Charcot-Leyden crystals. They arise in two ways, intracellularly and extracellularly. Most of the crystals arise within the cell. The crystals elevate, then puncture the cell membrane. More than one crystal may be formed within a cell. It is the usual picture with the

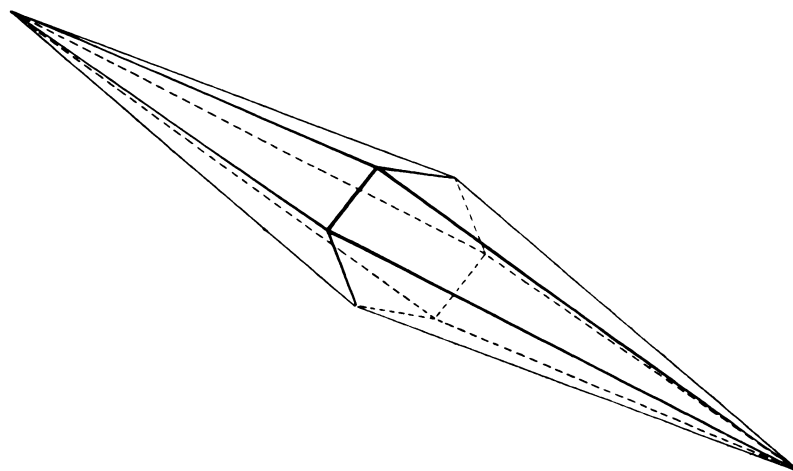


FIG. 3.—DIAGRAM OF CHARCOT-LEYDEN CRYSTAL

Aerosol MA method to see almost every eosinophil with one or more Charcot-Leyden crystals protruding from the cell. Often the crystals lie alongside the cell. Those crystals that arise extracellularly, first appear as minute crystals which gradually increase in size.

It is significant that, with the exception of the eosinophil granules and the Charcot-Leyden crystals, all formed elements of the blood are lysed, indicating that these granules and crystals are remarkably resistant to reduction in surface tension. The nucleus of the eosinophil is dissolved by Aerosol MA, since even after brief exposure to Aerosol MA, the nucleus of the eosinophil appears washed out and does not stain with Wright's stain. In control preparations, even after several days, some chromatin of the nucleus of the eosinophil takes the stain. There is no apparent reduction in eosinophil granules, nor change in their staining reaction, indicating that the crystals do not arise from the granules. It is probable then that the Charcot-Leyden crystals arise from the nucleus of the eosinophil, although this has not been proved.

It was stated by Schwarz⁶ that the Charcot-Leyden crystal is not an essential

component of the eosinophil. Thus, Brown⁹ studied the blood of a patient with trichiniasis who had 68 per cent eosinophils and was unable to produce Charcot-Leyden crystals. In our work, however, whenever eosinophils were found in blood



FIG. 4.—CHARCOT-LEYDEN CRYSTAL IN WET PREPARATION. X900

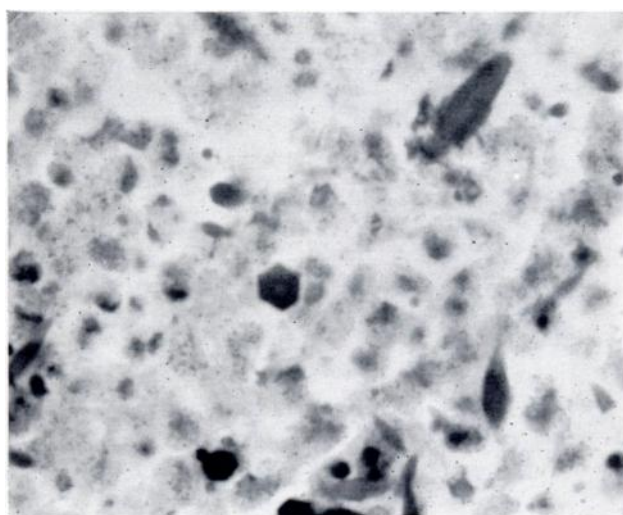


FIG. 5.—CROSS SECTION OF CHARCOT-LEYDEN CRYSTAL, SHOWING HEXAGONAL SHAPE. X900

or in tissue, they could be made to produce Charcot-Leyden crystals with Aerosol MA. Conversely, no crystals were found in blood or tissue in which eosinophils were absent. Also the number of crystals formed was directly proportional to the

number of eosinophils present. Preliminary work indicates that the Charcot-Leyden crystal is not only an essential component of the eosinophil, but it is also specific for the eosinophil.

In patients with considerable eosinophilia, the crystals may be demonstrated in whole blood. The technic is the same as described, except a drop of blood obtained from a small wound of the finger is used instead of the buffy coat. In one patient with eosinophilia of 60 per cent, of unknown origin, associated with transient erythematous swellings of the subcutaneous tissues, crystals of large size could be demonstrated in the peripheral blood in a few minutes by use of Aerosol MA.

Another patient with embryonal carcinoma of the testicle metastatic to the lungs was studied. He had a pleural effusion containing about 95 per cent eosinophils.

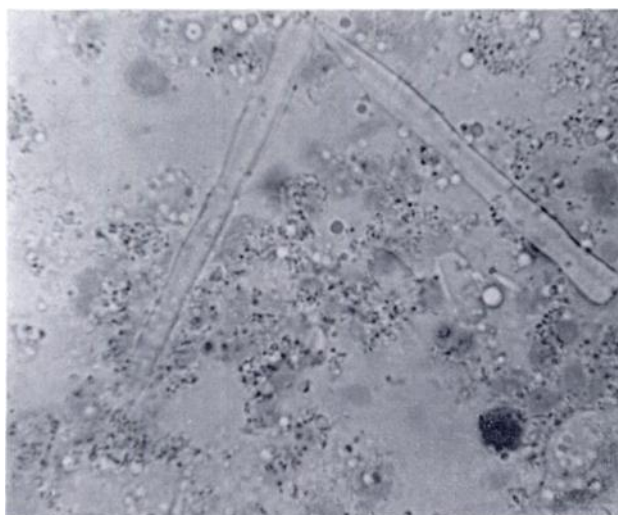


FIG. 6.—CHARCOT-LEYDEN CRYSTALS, SHOWING BLUNT ENDS. X900

The centrifugate of this fluid was then almost pure eosinophils. When this centrifugate was mixed with Aerosol MA, very large abnormal crystals with blunt ends were formed, together with normal crystals. The largest of the crystals measured 96 microns in length and 9 microns in width (figure 6). It is believed that the malignancy was not responsible for the formation of the abnormal crystals. The more logical explanation is that the substance which forms Charcot-Leyden crystals was here present in such a large amount and subject to the influence of Aerosol MA that unusually large and abnormal crystals were formed.

A study was made of a patient with discoid lupus erythematosus of the face of thirty years' duration during an acute exacerbation. He showed an eosinophilia of 36 per cent. The buffy coat of the blood of this patient when mixed with Aerosol MA formed crystals within two minutes, the largest of which measured 200 microns in length. This represents about ten times the usual size of Charcot-Leyden crystals. Some of these crystals were remarkable in that they were fused in their

centers. The control preparation showed crystals in two days. On treatment with Bismarsen intramuscularly, the patient rapidly improved, the rash almost entirely disappeared, the eosinophils decreased to 6 per cent and lost their ability to form Charcot-Leyden crystals rapidly and of the abnormally large size. The inference is that some test based on the size and time of appearance of the crystals may be evolved by which the progress of allergic diseases can be determined.

DISCUSSION

Reports in the older literature repeatedly emphasize that for the production of Charcot-Leyden crystals, the preparations must stand for a considerable period of time. It is also emphasized that large numbers may be found in the bone marrow of cadavers showing autolytic changes. In these cases there is almost always growth of bacteria and the release of ferments by the destruction of cells during autolysis.

It is believed then that the mechanism for the formation of Charcot-Leyden crystals relates to the destruction of the eosinophil by these various lytic agents. In this work, the control preparations did not become positive until the bacterial growth was quite heavy, and the majority of red cells and white cells showed degenerative changes. It is then believed that the mechanism of the action of Aerosol MA is related to its marked lysing effect due to its ability to lower surface tension. The conflicting reports in the literature then become understandable; in those cases in which the eosinophils were subject to lytic influences either by bacterial or enzymatic action, large numbers of crystals were produced, while the reverse happened if they were not subject to such influences. By the use of Aerosol MA these lytic factors may be controlled, with production of crystals from eosinophils in all tissues in which eosinophils were found.

Of particular interest is the work of Turner et al.¹⁰ on the relationship of the eosinophil to the Gordon phenomenon. They showed that the agent producing encephalitis in rabbits was found only in the presence of the eosinophils. They also showed that the test was only positive in those cases of Hodgkin's disease in which eosinophils were found in the tissue. The exact nature of the exciting agent is unknown. Turner and his co-workers suggest that it is related to the Charcot-Leyden crystal. Support for this theory is that for the production of the agent, the tissue must autolyze in the refrigerator for 1 to 2 weeks to obtain a positive test—a factor favoring the production of Charcot-Leyden crystals.

At present this method of producing Charcot-Leyden crystals by means of Aerosol MA has no practical significance. It should simplify, however, the isolation of the crystals, the determination of their chemical structure, and thus lead to a better understanding of the eosinophil and the diseases with which it is associated.

CONCLUSIONS

1. A method using Aerosol MA is presented by which Charcot-Leyden crystals may be formed from eosinophils with certainty, rapidity, and in quantity.
2. With the Aerosol MA method Charcot-Leyden crystals were demonstrated in 99 percent of the blood of 100 routine hospital admissions; the crystals were demonstrated in 80 per cent of the control group. With the Aerosol MA method

Charcot-Leyden crystals were demonstrated in 90 per cent of normal persons; the control group was positive for crystals in 58 per cent.

3. Preliminary work indicates that the Charcot-Leyden crystal is not only an essential component of the eosinophil, but it is specific for the eosinophil.

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REFERENCES

- ¹ CHARCOT, J. M., AND ROBIN, C.: Observation de leucocythemie. *Mem. de Soc. de Biol.* 5: 44-50, 1853.
- ² LEYDEN, E.: Zur Kenntnis des Bronchial-Asthma. *Virchows Arch. f. path. Anat.* 54: 324-352, 1872.
- ³ LIEBREICH, E.: In-vitro Versuch über Eosinophilie. *Klin. Wchnschr.* 2: 194-198, 1923.
- ⁴ NEUMANN, A.: Ueber die Natur der Charcot-Leyden-Böttcher-Neumann Krystalle. *Ztschr. f. phys. Chem.* 173: 69-71, 1927.
- ⁵ THOMPSON, J. H., AND PADDOCK, F. K.: The significance of Charcot-Leyden crystals. *New England J. Med.* 223: 936-939, 1940.
- ⁶ SCHWARZ, E.: Die Lehre von der allgemeinen und örtlichen "Eosinophilie." *Ergebn. d. allg. Path. u. path. Anat.* 17: 137-789, 1914.
- ⁷ HARRISON, G. A.: *Chemical Methods in Clinical Medicine.* New York, Grune & Stratton, 1947. P. 544.
- ⁸ BEELER, E. C.: Hydrophilic Ointments and Bases. I. Compilation of newer ingredients with an index to trade names. *Bull. Nat. Formulary Committee* 10: 173-232, 1942.
- ⁹ BROWN, R. R.: Experimental studies on the origin of Charcot-Leyden crystals with an account of their chemistry, occurrence, and diagnostic importance. *Philadelphia M. J.* 2: 1076, 1898.
- ¹⁰ TURNER, C. J., JACKSON, H., AND PARKER, F.: The etiologic relation of the eosinophil to the Gordon phenomenon in Hodgkin's disease. *Am. J. M. Sc.* 195: 27-32, 1938.