During class demonstration of perfusion or excretion experiments, it has been found that the sound of the relay is a valuable adjunct to the visual record made by the signal magnet. If the relay itself does not click loudly enough, a telegraph sounder may be incorporated in the secondary circuit. Changes in rate of flow are easily impressed on the class by this method.

By using a signal magnet wound to 1000 ohms, the relay and lamp may be omitted from the circuit and the contact points connected directly in series with the signal magnet, using a 22½-volt B battery as the source of current. This latter circuit is not advised for general use inasmuch as it requires a specially made and rather delicate signal magnet. This can be made in the style of the Harvard signal magnet from the magnets of a radio headphone, substituting for the permanent horseshoe magnet of the phone a short bar of soft iron.

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Variations in Monocytic Response to Peroxydase Test.

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Naegeli[1] states that almost all, if not all, of the human monocytes are peroxydase reacting. The author[2] found no peroxydase-reacting monocytes in the blood of a rabbit. In a normal adult man there were present 3.4% peroxydase mononuclear phagocytes and 1.1% phagocytes that did not react to the peroxydase test. The conclusion was that the peroxydase cells of human blood are of myeloid origin, which is the view held by Naegeli. The negative phagocytes were thought to be the true monocytes derived from the reticular portion of lymphoid tissue. Recently some unpublished observations have brought me to a consideration of the alternative view, namely, that the peroxydase-positive phagocyte gets its granulation secondarily in a more or less accidental manner. The conclusions arrived at in this paper are based on tissue cultures of blood leukocytes.

Methods and Results: Numerous cultures of human blood were made by the coverglass method and examined by the peroxydase test

[1] Naegeli, O., *Blutkranheiten und Blutdiagnostic*, Berlin, 1923.

[2] McJunkin, F. A., *Arch. Int. Med.*, 1923, xxxvi, 799.

daily from one day to 4 weeks. This was done on the assumption that the peroxydase granulation of the monocytes would disappear, provided the granulation was acquired secondarily and was not an essential part of the cell cytoplasm. After one month in the incubator large peroxydase reacting phagocytes were present in the cultures. Never was the number of phagocytes great and no conclusion was possible in regard to the proportion of positive and negative cells. Rhoads and Parker[3] found at the end of 2 weeks of incubation both positive and negative mononuclear phagocytes in normal human blood. The next procedure was to culture rabbit leukocytes in contact with variable amounts of human blood: Rabbit blood was cultured on sterile cover-glasses covered with smears of human blood made with aseptic precautions. The smears were kept dry in the air for 2 hours. The rabbit blood was obtained from the heart and drops of blood placed directly on the covers from the small caliber needle. The covers which had previously been rimmed with sterile vaseline were inverted over the well and placed in the incubator within 5 minutes after withdrawal of the blood from the heart. After about one hour the covers were sealed permanently with paraffin. At the end of 2 weeks as many as 500 large mononucleated cells were present in some of the cultures while in the human blood cultures mentioned above the cells at the most numbered a few dozen. Hundreds of the rabbit blood cultures were examined by the peroxydase method after 1 to 21 days of incubation: cover-glasses removed and covered for 30 seconds with 2 drops of benzidin (100 mgm. of dry benzidin, 20 cc. pure methyl alcohol, 5 cc. distilled water, 1 drop hydrogen peroxide) and then diluted with 4 drops of 1/10% copper sulphate. After a reacting time of about 2 minutes the cover is washed in distilled water made slightly acid with acetic acid, stained in hematoxylin (Harris) for one minute and again washed in the acidulated water in order to color the nuclei a purple so as to contrast with the green peroxydase granules. The preparations are blotted, dehydrated with acetone, cleared in xylol and mounted in balsam. The method is one used by the author[4] since 1920 except the dilute copper sulphate is substituted for distilled water for dilution. Copper sulphate was recommended by Sato and Yoshimatsu.[5]

Although in the usual smear of human blood kept dry at room temperatures for 2 or 3 days the peroxydase reaction becomes

[3] Rhoads, C. P., and Parker, F., *Am. J. Path.*, 1928, iv, 271.

[4] McJunkin, F. A., *J. Am. Med. Assn.*, 1920, lxxiv, 17.

[5] Sato, A., and Yoshimatsu, S., *Am. J. Dis. Child.*, 1925, xxix, 301.

greatly diminished it is well retained for 5 days in the cultures in the parts of the film not covered by the droplets of rabbit blood. In fact, the neutrophilic granules swell to become more conspicuous than in the fresh dry smear. By the twelfth day the granules have become indistinct. The best preparations have been obtained after 5 to 6 days of incubation. At this time there may be present almost as many large mononuclear cells in the peripheral zone as there are rabbit polynuclears. Many of them contain phagocyted nuclear material and in an occasional rabbit phagocyte there are peroxydase particles permitting certain identification. The greenish color of the granules makes possible a differentiation from nuclear particles and from hemoglobin. Sometimes the granules correspond in size to those of the human neutrophile while others have been seen in which the reacting cytoplasm was in a mass suggesting a portion of phagocyted human neutrophile. Ameboid rabbit monocytes have been seen in contact with human polynuclears. The presence of phagocyted material in the cells serves to eliminate a possible confusion of rabbit monocytes with persisting human monocytes. The abundant hemoglobin becomes even more objectionable after formalin fixation. Work is now in progress to culture the rabbit phagocytes in plasma with elimination of the rabbit red cells.

The author wishes to express his indebtedness to Mr. Anthony J. Bell for assistance in the earlier experiments.

Conclusion: Rabbit monocytes negative to the peroxydase test may acquire a positive reaction when cultured on smears of dry human blood. Since rabbit monocytes in tissue cultures may by phagocytosis acquire a peroxydase content it seems possible that the mononuclear phagocytes of human blood may get their peroxydase granules by the same process.