Tangential sections of mouse, rat, rabbit and cat kidneys, starting from the cortical surface and proceeding toward the medulla show that the juxtaglomerular corpuscles are absent in the superficial layers of the cortex corticis, but increase markedly toward the middle part of the cortex and decrease again toward the corticomedullary junction. They seem to be intrinsic characteristic structures of the normal, functioning mammalian kidney. The distribution of the macula densa in the kidney parallels that of the juxtaglomerular corpuscles.

It is important that the tissues be fixed while very fresh, preferably in Bouin's or Zenker's solution. They may be embedded in paraffin in the usual way and sectioned serially, if possible at 4 to 6 microns. Preparations stained with hematoxylin-eosin show the structures clearly, but do not give adequate cytologic details. Masson's trichrome stain is more satisfactory and Mallory's phosphotungstic acid-hematoxylin reveals good nuclear detail. Cytoplasmic vacuolization is clearly visible with this stain and with Mallory's anilin blue connective tissue stain. Intercellular fibrils are well brought out with Masson's stain or better with silver stains.

No definite suggestion as to the nature, the biological or physiological function of these cells can yet be given. Their intimate relationship with the macula densa is noteworthy and may suggest a physio-biologic interrelation between these two structures.

11411

Influence of Neoprontosil on Migration of Blood Leucocytes in Tissue Cultures.*

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Sulfanilamide and Neoprontosil have been reported to stimulate phagocytosis of bacteria by leucocytes *in vitro*. Finkelstein and Birkeland[1] found that in the presence of sulfanilamide and Prontosil (Neoprontosil?) the number of guinea pig leucocytes taking up bacteria and the number of bacteria engulfed per leucocyte was markedly increased. Fresh plasma or serum appeared to be neces-

* The Neoprontosil was furnished by the Department of Medical Research, Winthrop Chemical Co., Inc.

1 Finkelstein, R., and Birkeland, J. Y., *Science*, 1938, **87**, 441.

sary in order to obtain greater phagocytic activity in the presence of the drugs.

Gay, *et al.*,[2] however, found no definite difference in the *in vitro* phagocytosis of streptococci by exudate cells from sulfanilamide-treated rabbits as compared with normal rabbits. Streptococci that were treated with sulfanilamide were more readily phagocytized than non-treated streptococci.

Tunnicliff[3] observed that both sulfanilamide and Neoprontosil increased the phagocytosis of streptococci by blood leucocytes. She states that "By comparing the amount of phagocytosis in the leukocytes suspended in salt solution with that in leukocytes suspended in dilute prontosil-soluble the stimulating action of prontosil-soluble was observed to be on the leukocytes."

When Neoprontosil in 1:1000 concentration was added to whole blood with bacteria, the number of cocci ingested per leucocyte was doubled. Serum from mice receiving molar equivalents of sulfanilamide and Neoprontosil increased the phagocytic activity of normal leucocytes to the same degree.

King[4] demonstrated that sulfanilamide 1:1000 stimulated the rate of migration of rabbit leucocytes in sterile tissue cultures. After 24 hours of incubation the average migration rims were 18% wider than in the controls. The absolute difference was 5.8 times the standard error of the difference.

The influence of Neoprontosil (the disodium salt of 4' sulfamidophenyl-2-azo-7 acetylamino-1 hydroxynaphthalene-3, 6 disulfonic acid) on the migration rate of blood leucocytes in sterile tissue cultures was studied.

The routine culture methods used were described by King.[5, 6] To obtain the buffy coat, 15 cc of rabbit blood were drawn into a 50 cc centrifuge tube containing sufficient heparin to prevent clotting. The blood was centrifuged at high speed for 15 minutes and the plasma removed. The tube was recorked with a fresh sterile cork and re-centrifuged for one-half hour. If the tubes are handled carefully, the buffy coat can at the end of the second centrifugation be removed as a solid placque. After washing the buffy coat with Tyrode to remove the adhering red cells, it was fragmented and covered with Tyrode. Buffy coat fragments are extremely fragile. If allowed to

2 Gay, F. P., Clark, A. R., Street, J. A., and Miles, D. W., *J. Exp. Med.*, 1939, **69**, 607.

3 Tunnicliff, R., *J. Inf. Disease*, 1939, **64**, 59.

4 King, J. T., *Am. J. Physiol.*, 1938, **123**, 119.

5 King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

6 King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

remain in a fluid medium for more than one hour or handled roughly, they disintegrate rapidly.

The fragments, 2 to 3 mm in diameter, were carefully chosen in pairs according to size, shape and general texture. One fragment from each pair was used as the control, the other fragment as the experimental tissue. In this way each control culture has a visually identical experimental culture. Each series contained 30 cultures made from one animal.

The cultures were planted in moist chambers (Maximow technic) and incubated as lying drops in a special down-draft incubator described by King.[7]

The fragments were planted in one part autogenous heparinized plasma and 3 parts of an autogenous rabbit serum extract of 6-day chick embryos. Sufficient Neoprontosil was added to the serum extract to make a 1:1000 concentration in the final culture medium.

Observations were made on the living cultures at the end of 24 hours of incubation. The maximum migration rim was measured with a 16 mm objective and a 6× ocular with an eyepiece micrometer (114 units = 1 mm).

TABLE I.

No. of series	No. of cultures per series	Avg control	Avg experimental	Absolute difference	S.E. of difference	% increase
16	30	220	242.8	22.8	7.48	10.3

The experimental data are shown in Table I. From the results it appears that Neoprontosil 1:1000 increases the rate at which rabbit blood leucocytes migrate in tissue culture media. The absolute difference in the width of the migration rims between the experimental and control cultures is 22.8 and the standard error of the difference is 7.48.

Whether there is any significant correlation between the increased migration and the increased phagocytosis by leucocytes *in vitro* is not clear at present.

The possibility that the Neoprontosil might be partially split into sulfanilamide in tissue culture media and exert its stimulating effect as such must be considered. No experimental evidence is available on this point.

Conclusion. In tissue culture media composed entirely of animal body fluids Neoprontosil 1:1000 stimulates the rate of rabbit blood leucocyte migration.

[7] King, J. T., *Arch. f. Exp. Zellforsch.*, 1937, **20**, 208.