from cellular detritus. This suspension was prepared in such concentration that a 1:10 dilution corresponded to No. 2 of the McFarland scale, thus having an equal rickettsial content to our monovalent concentrated murine vaccine.

The theoretically "bivalent vaccine" was tested for specificity and potency by means of selective complement fixation tests[2] and by protection experiments in guinea pigs comparing the vaccine with a monovalent murine vaccine.

For complement fixation tests the "bivalent vaccine" was titrated with sera from guinea pigs bled after recovering from murine and classic typhus. The titration was made comparing the antigen with known monovalent murine and classic antigens prepared from the lungs of mice. Table I shows the results of tests made with monovalent and bivalent guinea pig sera and monovalent and bivalent antigens. It is apparent that the bivalent antigen acts as a mixture of murine and classic rickettsiæ.

According to previous experiments, the monovalent murine vaccine protected 3 out of 4 guinea pigs against classic typhus when the animals were vaccinated with a 1:10 dilution of the stock rickettsial suspension. For the comparative protection experiment we vaccinated guinea pigs with dilutions of the stock vaccines ranging from 1:20 to 1:50. Care was taken to have equivalent suspensions of monovalent and bivalent vaccines. Eight guinea pigs were vaccinated with monovalent vaccine and 8 with the bivalent rickettsial suspensions. Two animals were treated with each dilution, administering subcutaneously 2 doses of 1 cc each at weekly intervals. The vaccinated animals were inoculated with 0.1 g of the brain of a guinea pig infected with the "Breinl" strain of typhus, 18 days after the last dose of vaccine. Table II shows the results of this experiment, including 3 controls.

It may be seen in Table II that the temperatures of the animals vaccinated with "bivalent vaccine" show many more figures below 40°C than were found in the group of animals vaccinated with the monovalent murine vaccine. It is evident, therefore, that vaccines prepared in rats with mixtures of murine and classic viruses produce a better protection against classic typhus than the material obtained after the inoculation with murine virus alone.

The serologic and protection tests here described seem to indicate that the inoculation of mixtures of murine and classic viruses produce a mixed infection in which the classic virus seems to have an important part.

Summary. Typhus rickettsiæ of the classic type which have no tendency to produce massive infection in rats, seem to multiply more readily when associated with murine rickettsiæ. The animals inoculated by intranasal route produce "lung vaccines" of higher immunizing value against classic typhus than monovalent murine vaccines.

[2] Plotz, H., *Science*, 1943, **97**, 20.

14706

Distribution of Alkaline Phosphatase in Kidney Following the Use of Histochemical Azo Dye Test.

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The technic of a histochemical azo dye test for the demonstration of alkaline phosphatase in kidney has recently been described in detail by us.[1] The test consists of a direct coupling of calcium β-naphthol, following its release from calcium β-naphthol phosphate by renal

[1] Menten, M. L., Junge, J., and Green, M. H., *J. Biol. Chem.*, 1944, **153**, 471.

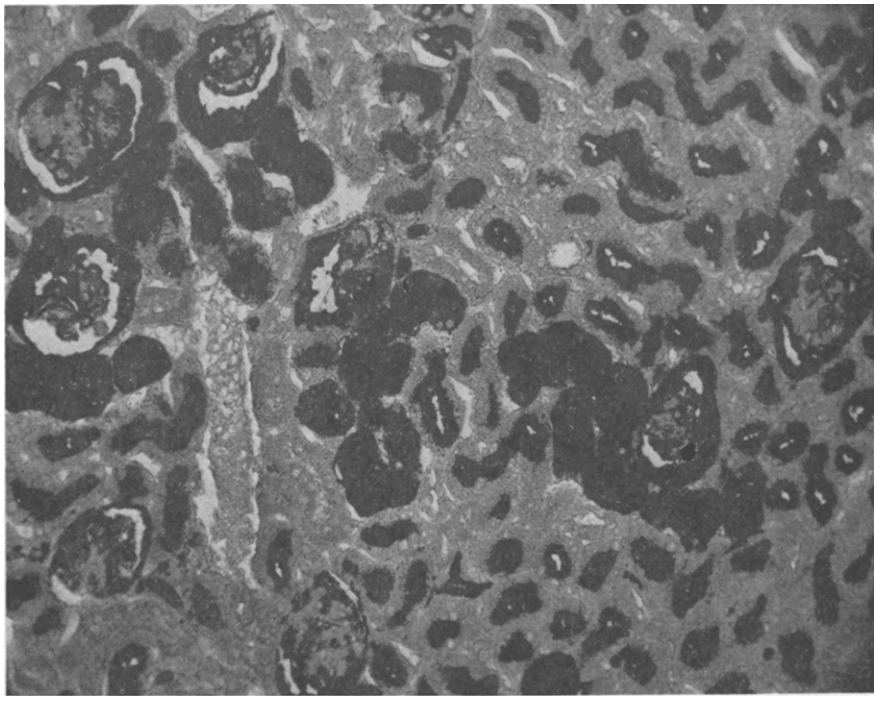

FIG. 1.
Mouse kidney: Dye deposit in glomerulus, capsular space, capsular epithelium, and the brush border and lumen of the proximal convoluted tubule. × 220.

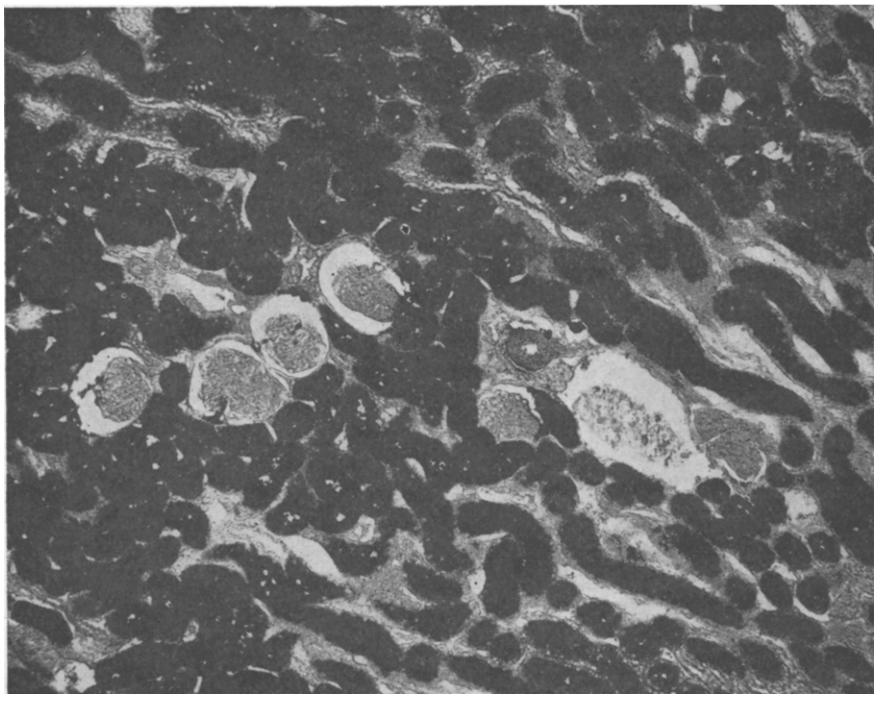

FIG. 2.
Rat kidney: Dye deposit in the brush border and lumen of the proximal convoluted tubule. A trace of dye occurs in some of the glomeruli. × 110.

phosphatase *in situ*, with a diazotized amine. This coupling forms an insoluble reddish purple dye. The loci of the precipitated dye in paraffin sections of kidney indicates the site of the phosphatase activity.

Mouse, rat, and human kidney were studied. The alkaline phosphatase was found mainly in the cortex where it occurred in greatest abun-

dance in the proximal convoluted tubules. The medulla, except in one or two instances, was free of enzyme.

In the nephron of the adult mouse, dye deposition began in the glomerulus in moderate amount, was slight in the flattened epithelium of the upper half of Bowman's capsule and reached a high concentration in the columnar lining of the lower half of the capsule. The capsular space also contained quantities of the dye. The dye extended down in maximal amount into the first part of the proximal convoluted tubule and gradually decreased toward the lower straight segment (Fig. 1). The remainder of the nephron contained little or no dye, with the exception of one instance, a mouse, in which the precipitate was abundant in the distal convoluted tubules. Details of distribution in the different parts of the nephron were of considerable interest. The greatest amount of dye occurred in the brush border and in the adjacent lumen. The beaded line stood out like a string of small reddish purple beads. The mitochondria in the epithelium of the first part of the proximal convoluted tubule frequently contained considerable dye. In the tuft the amount varied in individual animals, but was generally in greater concentration in the epithelium than in the endothelium. The endothelium appeared as an irregular wavy ribbon beneath which the basement membrane was well demarcated as a thin homogeneous red line. The juxta-glomerular body and the afferent arteriole often showed a moderate amount of diffuse hyaline pink color. Occasionally, other small arteries showed a similar coloration throughout their walls. The macula densa, where this structure could be identified, was relatively free of dye. In some sections a black precipitate occurred in sparsely distributed nuclei which possibly may have belonged to neutrophils. The nature of the black reaction is not known.

In the rat, the dye was limited to the cortex, occurring not only in the proximal but also in the distal convoluted tubules and with a variable deposition in many of the ascending limbs of the loops of Henle (Fig. 2). It was absent in the descending limb of the loop of Henle. The dye was seen in the epithelial lining of an occasional Bowman's capsule in moderate or slight amounts around the entrance to the neck. In the glomerular tuft, absence or only an occasional ill-defined trace of color was the rule. In the proximal convoluted tubules the precipitate was in the brush border and outer part of the lumen, and in the distal tubules in the lumen close to inner border of the cells. Rarely the granules of the mitochondria in the proximal convoluted tubules revealed the presence of dye (Fig. 3). Presence of phosphatase in the wall of the arteries of the medulla, as described by Gomori,[2] has been only rarely observed by us.

In the human kidney the distribution of dye corresponded more closely to that of the mouse kidney than that of the rat. The dye was slight or absent in the glomerulus and Bowman's capsule (Fig. 4). The amount of precipitate was moderate and was most pronounced in the first part of the proximal convoluted tubule, where it appeared in the brush border and in the lumen. The beaded line was distinctly shown as a granular red line. Occasionally, faint color was seen in the other locations observed in the kidney of the rat. The delay in obtaining autopsy material, as well as pathological changes in the human kidney, may modify the concentration of renal phosphatase, and probably accounts for our failure to confirm Gomori's[2] finding of the enzyme in the ascending loop of Henle and the cortical arterioles.

In parallel sections made from the same tissue as that employed in the azo dye technic and using glycerophosphate, phenylphosphate, and calcium β-naphthol phosphate in the Gomori test we have noted similarity of enzyme activity and distribution with the three substrates, with the possible exception of the calcium β-naphthol phosphate which required the addition of magnesium to bring out the whole activity of the phosphatase. It should be noted that the concentration of this ester was small due to its low solubility. These observations indicate that there is an identical location of the enzyme with both technics

[2] Gomori, G., *J. Cell. and Comp. Physiol.*, **1941, 17**, 71.

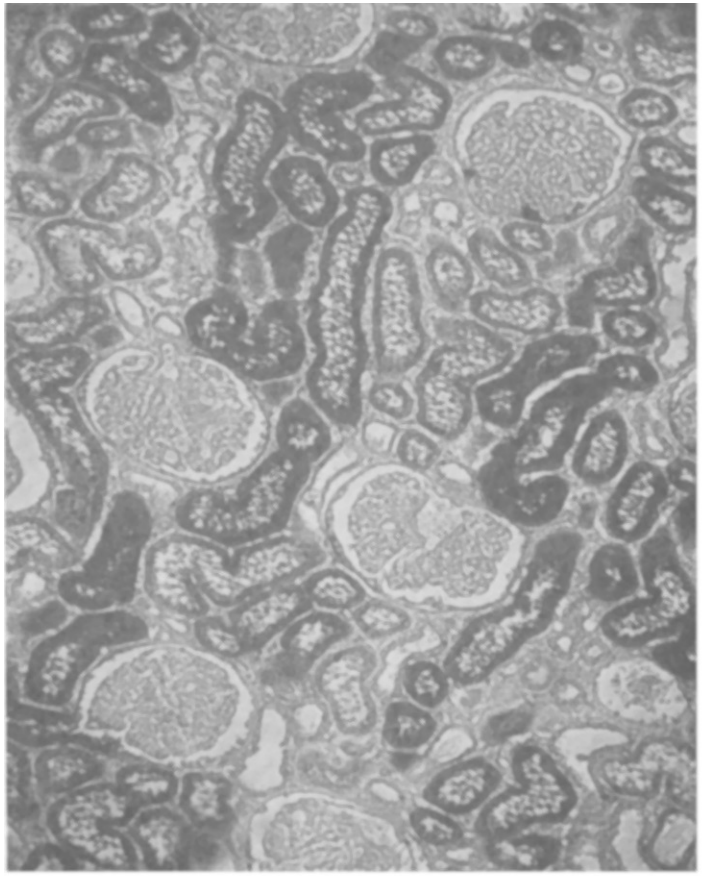

FIG. 4.
Human kidney: Dye practically limited to first part of the convoluted tubule. × 110.

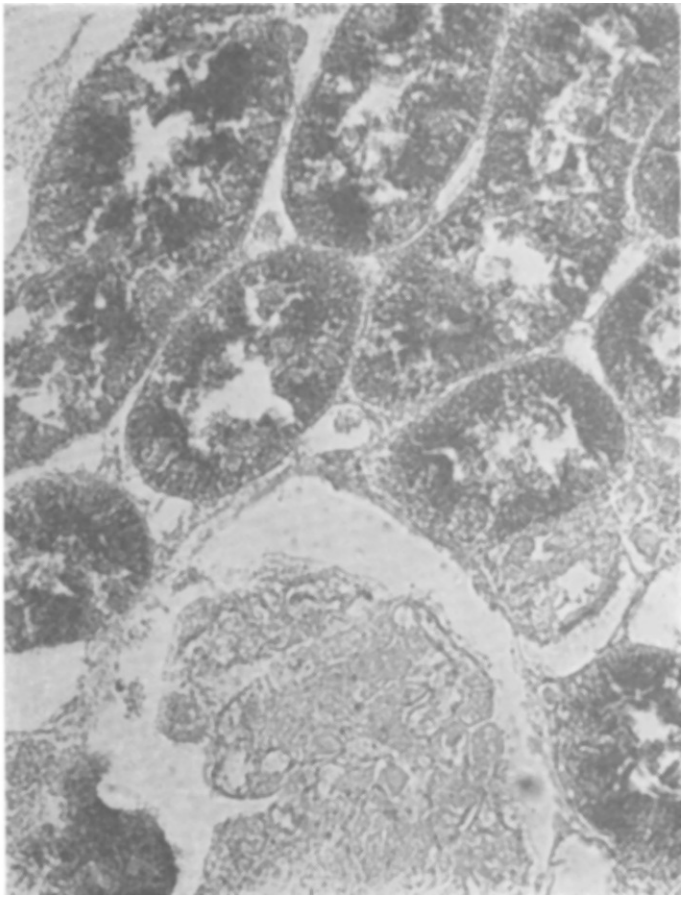

FIG. 3.
Rat kidney: Dye on the mitochondria of cells of the proximal convoluted tubule. × 700.

and also that a single renal monoesterase rather than multiple esterases is involved.

In general, our results confirm those reported with the Gomori[3] and the Takamatsu[4] technic. Minor differences, however, were observed, such as the presence of phosphatase in the glomerulus in contrast to its absence in this structure reported by Kabat and Furth.[5] In the study of a series of miscellaneous animals Wilmer[6] recently reported phosphatase in the glomeruli of the cat and the chicken, so that enzyme activity in this structure undoubtedly varies in different species.

It is interesting that the mouse is the only one of the 3 species studied by us in which the dye appeared in large amounts in the capsular lining which is uniquely of columnar type epithelium in this animal. The presence of the enzyme in this position, as well as in glomerulus, raises the question whether phosphatase activity and the metabolic processes with which it is associated may not be instituted at a higher structural level in the nephron of certain species than of others.

The occasional clear-cut demonstration of phosphatase in the mitochondria of the proximal convoluted tubules suggests that these cellular structures are functionally related to or are associated with the development of this enzyme in the kidney. Recently, Breedis, Flory, and Furth[7] and Wilmer[6,8] have noted that pathologic changes in the cytoplasm of the proximal convoluted tubules result in loss and disappearance of the phosphatase from their lumina. This observation gives support to the belief that the mitochondria may be the site of origin of the renal alkaline phosphatase.

Summary. The location of the alkaline phosphatase in the kidney of the rat, mouse, and human shown by the use of a histochemical azo dye test is mainly in the proximal convoluted tubule. This distribution agrees substantially with that obtained by the silver technic of other investigators. Minor differences in the enzyme distribution in the three species are described.

Thanks are due to Dr. Mortimer Cohen and Miss Ann Shiras for making the photomicrographs.

[3] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

[4] Takamatsu, H., *Tr. Jap. Path. Soc.*, 1939, **29**, 492.

[5] Kabat, E. A., and Furth, J., *Am. J. Path.*, 1941, **17**, 303.

[6] Wilmer, H. A., *Arch. Path.*, 1944, **37**, 227.

[7] Breedis, C., Flory, C. M., and Furth, J., *Arch. Path.*, 1943, **36**, 402.

[8] Wilmer, H. A., *J. Exp. Med.*, 1943, **78**, 225.

14707

Studies in Hodgkin's Syndrome. II. A Search for Brucella in Hodgkin's Syndrome.*

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The coexistence of Brucella infection and Hodgkin's syndrome has been reported by Parsons and Poston[1] and Forbus[2] in a series of 24 cases studied by them at Duke University. The strains of Brucella which were obtained induced a mild infection when inoculated into experimental animals. There was an atypical distribution of lesions described, from which Brucella were not regularly recoverable. Specific agglutinins could not be

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[1] Parsons, P. B., and Poston, M. A., *Southern Med. J.*, 1939, **32**, 7.

[2] Forbus, W. D., *Am. J. Path.*, 1942, **18**, 745.