

THE IMMUNOLOGICAL SIGNIFICANCE OF COLOSTRUM

III. INTRANUCLEAR BODIES IN RENAL DISEASE OF CALVES

By THEOBALD SMITH, M.D.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

PLATES 9 AND 10

(Received for publication, January 6, 1930)

The urinary organs of young calves are vulnerable, and pathological conditions readily appear in an abnormal environment. Of these the so-called spotted kidney has been discussed in an earlier paper.¹ In this condition evidence points to a sublethal infection with *B. coli*. The process involves both organs alike. The multiple white foci of infiltrated cells are situated in the cortex and are embedded in normal tissue. The process ends either in a reabsorption of the exudate cells or in sclerosis whereby the enclosed functional structures are destroyed. The urinary secretion contains only a trace of protein and the lesion is not recognized until the animal is killed. The kidneys are as a rule somewhat heavier than the normal organ.

Another pathological condition expresses itself in large, uniformly white kidneys symmetrically involved. The lesions of the spotted kidney may be present also. Cases of the white kidney were observed several times in calves reared in the usual way about 10 years ago. Latterly experiments involving the withholding of colostrum and the feeding of serum in its place have brought a few to the surface. Before discussing the probable nature and etiology of the condition a few cases will be presented. To begin with, a case will be described in which the presence of intranuclear bodies was associated with nearly normal organs.

*Calf 1532.*² Holstein male born Jan. 11, 1929. Received in 3 consecutive doses, up to the 13th hour, 1400 cc. of cow serum mixed with cow's milk. 700 cc. of the

¹ Smith, T., *J. Exp. Med.*, 1925, 41, 413.

² This calf is tabulated among others in *J. Exp. Med.*, 1930, 51, 488.

serum came from untreated cows and 700 cc. from a cow treated with *B. coli* filtrates (D) and found only feebly protective for guinea pigs.³ The serum had been stored for nearly 2 years. No significant amount of specific protective antibodies had thus been fed to this animal.

During the 2 months of life there were no disturbances except a brief period of liquid stools during the 2nd day. The urine following the heat test became faintly clouded throughout but there was no measurable deposit⁴ of coagulum except on one occasion when it was 1.3 per cent. The gain in weight was normal, also the temperature curve. It was killed by a blow and severing neck vessels when 2 months and 8 days old. The following represent the few deviations from the normal found at autopsy.

The lymphoid tissue of the small intestine was below the normal in quantity but the thymus was normal in weight (300 grams). In the upper portion of the duodenum the mucosa was dotted with roundish elevations which were later recognized in sections as a lymphocyte infiltration of the villi. In the cecum the veins of the submucosa were marked by pigment deposits. The same condition extended 15 or 17 cm. below the ileocecal valve. The free border of the valve was deeply pigmented. In the upper colon were two areas showing numerous vague whitish spots 1-2 mm. in diameter. In sections, these spots were shown to be lymphoid follicles in submucosa. They were pear-shaped and the tapering portion extended into the mucosa between the tubules. A small number had reached the surface in the form of cylindrical plugs. Over them the surface epithelium had disappeared.

The kidneys were about $1\frac{1}{2}$ times the average weight at this age (192 and 200 grams, respectively, with capsule stripped off). On section, faint pale radial striations could be detected in cortex. In frozen sections of the fresh tissue, the condition shown in Fig. 1 presented itself. In the columns of Ferrein the epithelium of the tubules contained irregular foreign bodies all located within the nuclei. Some were in cells of the convoluted tubules. They appeared as irregular agglomerations of 2μ discs, some 4 to 6 consolidated into a straight or bent line. The epithelium was not distorted or distended as yet. In scrapings of the fresh (unfrozen) tissue, the bodies were present, hence freezing had nothing to do with bringing them out. In sections of tissue fixed in Zenker and alcohol, they were not changed and were colored reddish in stains containing eosin. In general the kidney tissue appeared normal, with perhaps a very slight hyperplasia of the interstitial tissue. In Figs. 2 and 3 a thin film of scrapings of the cut surface stained in hematoxylin and eosin brings out the intranuclear position of these bodies. The cytoplasm of the epithelial cells has been broken up by the procedure and only the nuclei have retained their form.

³ For the method of testing protective power see *J. Exp. Med.*, 1930, 51, 474. For a brief description of the cows furnishing serum see *J. Exp. Med.*, 1930, 51, 483.

⁴ For the rough estimation of protein coagulum in urine see *J. Exp. Med.*, 1930, 51, 485.

This is the first and thus far the only animal in which the intraglobular bodies were found in mass under apparently still normal functioning of the kidneys and normal condition of the animal generally.

The following fatal case is perhaps typical of the uncomplicated syndrome. Death took place during the writer's absence and only the formalinized kidneys and Zenker-fixed portions were available.

Calf 1511.—Guernsey bull, born May 22, 1928. Fed 700 cc. serum of Cow C⁵ (treated with *B. coli* mutant 1192_b) mixed with milk, in 3 doses as heretofore, the last dose when calf was about 13 hours old; thereafter only milk.

There were no disturbances of noteworthy extent during the 1st month. It was turned out into a paddock at this time and fed alfalfa in addition to the milk diet since it refused hay and grain. During the 2nd month it became unthrifty and constipated, the coat rough and abdomen distended. July 19, the calf was found down. It took milk very slowly. There was present tenesmus with passage of small amounts of feces. On July 20, it took a little milk early and was found dead at noon. The temperature of this calf taken twice daily was within normal range throughout if we except a slight rise on the 4th, the 27th–31st, and the 46th day.

*Autopsy.*⁶ Mucous membranes pale. Subcutaneous tissues around buttocks and scrotum edematous, the fluid, blood-tinged. Both kidneys enclosed in a layer of edematous tissue. The left is also enclosed in a blood clot. The bladder and rectum are surrounded by edematous, blood-tinged tissue. On dorso-ventral tip of spleen a projecting, flattish, hemorrhagic elevation. When the kidneys are shelled out of the edematous envelopes, they are found larger than normal. The weights are for the left kidney 540 grams, for the right 460 grams. This is between 2 and 3 times the weight of normal kidneys at this age. Both appear uniformly white with numerous hemorrhagic points near surface from $\frac{1}{2}$ to 2 mm. apart. On section, cortex whitish throughout with broad injected vessels radiating from the medullary boundary into both cortex and medulla.

The progress of the renal disease was reflected to a certain extent in the urine. Up to and including the 39th day of life there was a faint clouding when it was acidified and boiled. No further samples were tested until the day before death when the coagulum formed after heating was equivalent to 22.6 per cent in volume after 24 hours' sedimentation. Urine taken from bladder at the autopsy had the same volume of coagulum.

The kidneys hardened in Zenker's fluid and formalin were available for further study. Transections of the entire organ indicated some interference with the outflow since the calices were separated by a distinct space from the papillae. Stasis was further indicated by a general distention of the convoluted tubules

⁵ See *J. Exp. Med.*, 1930, 51, 484.

⁶ By Dr. R. B. Little in the writer's absence.

beginning at or within the glomerulus. The cortical structures were also driven apart by edema. The interstitial connective tissue was slightly increased and this was moreover evidenced by occasional mitoses. Within the nuclei of the epithelium of the convoluted tubules were inclusions, in form disclike, about 2μ in diameter (Figs. 4 and 5). The number of discs in a nucleus varied up to 12. They were fused together in an irregular, longitudinal mass. As a result some cell nuclei were greatly distended. One measured 15μ by 20μ . Any definite geometrical form was not recognizable except the generally circular outline of the discs. The fused masses have sharp borders, homogeneous surface, and suggest crystals. The intranuclear situation is easily recognized except when the masses are largest. Even then the greatly distended nuclear membrane may be made out. They are found in the labyrinth and the columns of Ferrein and quite irregularly in these regions.

The hemorrhages are of two kinds, those starting from glomeruli and those about the loops of Henle. Scattered about are groups of hemin crystals. Hyaline casts are found in the smaller collecting tubules and Henle loops.

In the hardened tissue, in cross sections of some of the distended tubules, there are from one to all of the epithelial cells with intranuclear bodies. The involved cell usually projects well beyond its neighbors into the lumen. Rarely a group of two or three hypertrophied cells shed into the lumen nearly occludes it. In the medulla, occasional greatly dilated collecting tubules are encountered. In others, hyaline casts are present. Associated with these inclusions there is hypertrophy and some degeneration of cells in both primary and secondary convoluted tubules and moderate interstitial edema together with some cell infiltration probably monocytic. The striking feature is the general distension of tubules from Bowman's capsule down.

Some tests with acids and alkalis were made with frozen sections of the formalinized kidneys. Thus 4 per cent NaOH acting upon the sections for 18 hours caused no change in the inclusions. Similarly 5 per cent acetic acid failed to dissolve them, although they seemed a trifle smaller.

The following is another typical case.

Calf 1411.—Holstein male, born Jan. 12, 2.40 p.m. Receives 400 cc. serum of Cow D (*B. coli* filtrate) plus an equal quantity of milk. At 8 hours it receives 200 cc. and at 15 hours 100 cc., also with milk added; thereafter only milk. This animal passed through a relatively severe attack of liquid stools which lasted 5 days. Later, traces of protein appeared in the urine from time to time. On March 4, the urine was clouded with fine flocculi which were covered with masses of small diphtheroid rods. Cultures brought to light *B. pyogenes* and the specific cystitis bacillus.⁷

⁷ Jones, F. S., and Little, R. B., *J. Exp. Med.*, 1926, **44**, 11.

March 23. Calf tends to lie down. Grits its teeth. Takes only a portion of the offered milk. Temperature about normal.

March 30. Calf has been growing weaker and taking only about one third the normal amount of milk. Marked constipation. Knee joints weak and evidently painful but not swollen. Urine contains a coagulable deposit equal to one third the bulk of entire fluid. Animal killed and autopsied.

The following abnormalities were noted. The rumen contains a very large quantity of hair intimately mixed with the semisolid vegetable contents. The fourth stomach contains a viscid white fluid and some twine from the bedding. Some petechiae in the leafy portion. In the large intestine, feces are dry and formed into hard pellets.

The perirenal fat is edematous, and from $\frac{1}{2}$ to 1 cm. thick, owing to the accumulation of a clear colorless fluid in its interstices. The capsule easily removed. The cortical tissue is very pale, without normal markings and dotted with dark red points, about 1 mm. or less apart. Under capsule are scattering hemorrhages and clots, several in each lobe and from a few to 15 mm. in diameter. On section, the same dark red points throughout cortex. Medulla without noticeable changes. Kidneys, after removal of fat, weigh each 350 grams, *i.e.* they are nearly twice normal weight.

Free hand and frozen sections of these organs show lesions of the epithelium of the convoluted tubules. Within the nuclear membrane is a group of fused disclike bodies of moderate refringence and slightly brownish tint. The individual discs composing the mass are 1 to 2μ in diameter. The masses themselves 5 to 10μ in diameter. Besides these intranuclear bodies there are larger masses of similar material irregular in form gathered in lumina of tubules and blocking them in part or wholly. 5 per cent acetic acid does not disturb them. Normal NaOH acts upon them and after several hours reduces them to heaps of minute granules.

Sections of kidney tissue fixed in Zenker's fluid show a marked irregular dilatation of the convoluted tubules. Nuclear membrane of the epithelial cells irregularly folded, one portion pressed toward the opposite half. They are 2 to 3 times normal size and contain faintly stained foreign masses. The glomeruli are either normal or else contracted and pressed to one side. The glomerular space is filled with a bubbly mass or else with red corpuscles. Hemorrhages are widespread in convoluted tubules, with the glomeruli as the probable source of the blood. The columns of Ferrein appear more compact than is normal, suggesting slight hyperplasia of the stroma. Hyaline casts present. In the collecting tubules hyaline casts numerous.

In certain animals showing renal lesions intranuclear bodies were not detected. However, there were observed casts either in the urine or in the collecting tubules made up of apparently the same material as that within the nuclei of the epithelial cells. Details of these cases are omitted.

An illustration of the association of the lesions of the spotted kidney with the type under discussion is furnished by a calf⁸ which received colostrum after a delay of 22 hours. The kidneys of this animal closely resembled those of No. 1511. Intranuclear bodies were present and located chiefly in the labyrinth.

In the course of experiments⁹ on the substitution of serum for colostrum a certain number of calves died unexpectedly in the 2nd month of life during the night or quickly after convulsive seizures during the day. In one of the animals thus affected intranuclear bodies were found. The relation between this acute fatal affection and the more slowly developing renal lesions is an open one. There are, however, reasons for tentatively regarding the renal and the convulsive type as allied etiologically. The following case among several is therefore briefly outlined.

Calf 1416.—Holstein heifer, weighing at birth 70–75 lbs. It received in place of colostrum during the 1st day 700 cc. of serum of Cow B (treated with heated *B. coli*). Slight proteinuria on 2nd day disappearing on 3rd day. On the 50th day of life, the calf refuses part of the usual amount of milk and develops symptoms due to irritation of central nervous system. The left ear is held back against head. The calf moves at times in a circle with unsteady gait. The left side of head and trunk is pressed against the sides of stall leading to contusions of head on that side. It is evident after a time that the calf fails to see. The pupils are dilated. Later in the day the animal lies down, with head drawn to the left and right ear twitching. Gets up and walks in a circle. Profuse sweating. The next day calf is unable to swallow its food. Gait unsteady. Forelegs spread apart. Knees and ankles bent. The symptoms remained unchanged until the 4th day. The calf began to take milk from a bottle. Blindness continued. During these days the urine contained about 1 to 1.5 per cent coagulable deposit and the temperature did not rise above 39.5°C. The case was tentatively considered one of botulism poisoning. The specific bacillus could not be demonstrated in the fecal discharges. Constipation was marked and the feces were in the form of small balls.

97th day. Calf has remained blind, but has recovered in other respects. Urine has contained from 1 to 3 per cent coagulable material up to the present. Killed today. The abnormal conditions noticed are as follows:—Digestive tract normal except lower half of large intestine which is filled with very dry fecal balls 1 to 2 cm. in diameter. Slight, rather firm adhesions of right cephalic and ventral lobe of lungs to ribs. Collapse of scattering lobules in cephalic lobe. Both kidneys en-

⁸ Smith, T., *J. Exp. Med.*, 1925, 41, 422.

⁹ Smith, T., and Little, R. B., *J. Exp. Med.*, 1922, 36, 453.

larged and with uniformly pale cortex, increased in thickness. Medulla sharply demarcated from it by fullness of vessels. Weight of each kidney, freed from fat envelope, about 305 grams.¹⁰ Some slight adhesions of caudal border of liver to kidneys and other structures. A trace of fat in liver cells. Malpighian bodies of spleen very distinct. Ovaries cystic. Brain and medulla without visible abnormalities.

In free hand and frozen sections of kidneys, the epithelium of the convoluted tubules contains the same kind of material described under Calf 1411. There are no casts of this material in the lumina.

Examination of fixed material from kidneys shows a slight distension of convoluted and straight tubules in limited areas throughout cortex. Scattering interstitial foci of cells made up of polynuclear and some mononuclear leucocytes. The enveloped tubules filled with polymorphs. Epithelium of convoluted tubules with enlarged and irregularly folded nuclei, appearing like collapsed spheres. In certain areas there are intranuclear discs stained reddish. Casts present but in small numbers. Sections of liver, spleen and brain without noticeable abnormalities.

This is the only animal to survive the acute attack. The renal lesions resembled those of the group in which such an attack was not observed. However, in several acutely fatal cases the highly congested kidneys did not show intranuclear bodies.

DISCUSSION

The observations set down in the preceding condensed protocols are published at this time because of the interest manifested in similar structures associated with certain infectious diseases and also because the investigations have been discontinued for the time being. The material is relatively scarce, it has been available at unexpected, inopportune moments, and in certain instances it was undiagnosed during life. It nevertheless contains a certain interest in that it presents several significant problems in renal pathology.

As regards clinical indications it may be said that they were relatively scarce. Proteinuria was present only towards the end of life. The amount of protein was then quite large. Constipation was marked in several animals. The temperature rarely rose more than a degree C. and usually in association with diarrheal conditions.

The intranuclear bodies were observed both in an otherwise still

¹⁰ Normal weight at this age about 200 grams.

normal kidney and in organs markedly altered. The chief characters are enlarged white organs covered with an edematous layer involving the perirenal fat. The cortex is peppered with petechial hemorrhages which on section are seen to be present throughout the cortex. The distension of the entire convoluted tubule system of the labyrinth was present in some, and of only certain portions in others. Isolated collecting tubules were also greatly distended. In the formalin-fixed kidneys, the calices of the pelvis were distended. Inflammatory reactions, such as interstitial focal collections of cells and hyperplasia of stroma, may or may not be present. Hyaline casts are frequently seen, chiefly in Henle's loops. Bacteria are absent both in cultures and in sections of the organ.

The intranuclear bodies are in all cases alike. They are made up of roundish disclike units, 1 or 2μ in diameter, fused into masses containing up to a dozen discs. The fused masses are irregular in form and suggest concretions. Weak acids and alkalies have not dissolved or otherwise changed them materially. They are situated mostly in the epithelium of Henle's loops, more rarely in the convoluted tubule epithelium. These bodies are not of common or frequent occurrence in the calf. The kidneys of many calves killed after 2 months and apparently normal were searched in vain for these bodies. A study of the tables in another publication¹¹ brings out the fact that the renal lesions were found only in calves in which there was a deviation from the normal colostrum feeding, either by substituting serum, or by delaying the intake of colostrum, or allowing only a small amount of colostrum at the start. However, only a small per cent of these were affected. It is significant that none of the 14 controls¹¹ allowed to feed normally with the dam for 3 or 4 days were abnormal when kept 4 to 10 weeks.

Thus far no information bearing on the nature of the intranuclear bodies is at hand. Their appearance suggests some form of concretion, deposited or formed within the nuclear membrane. The involvement of the cells may be quite extensive, almost universal, as in No. 1532 (Figs. 1-3).

The writer has been unable to find in medical publications references

¹¹ Smith, T., and Little, R. B., *J. Exp. Med.*, 1930, **51**, 483.

to renal conditions similar to the ones described, except a paper by Van Leersum.¹² In studying urinary calculi in rats on a vitamin-A-deficient diet he came to the conclusion that the calculi are not merely deposits of urine salts but made up of "epithelial cells impregnated with calcium." He did not, however, trace the process back towards its beginnings in the attached cell.

The effect on the epithelial cells is at first almost negative. Later the cells become hypertrophied as the inclusions grow and project into the lumen of the tubules. In one case a mitotic cell was detected in which the inclusion had been pushed into the cytoplasm. The cell was thus still capable of reproduction. Whatever the final effect, the degenerative process appears to be slow. The end result, as suggested above, is probably the shedding of the cell and its replacement by mitotic multiplication at least for a time.

Taking the various gross and microscopic data into consideration, we may conclude that the main disturbing factor is occlusive and that occlusion is due to a shedding of the involved epithelium with the formation of casts in the tubules and possibly later on concretions obstructing also the ureter. The perirenal edema and multiple petechial hemorrhages favor this theory. The presence of numerous casts in certain cases not here reported made up of dislike bodies is contributory evidence. It is conceivable that in the animal (No. 1532) in which the epithelium of the still normal kidneys was universally involved, the simultaneous discharge of these concretions might cause blocking of the entire excretory system. Complications of this type of injury, with lesions due to the "spotted kidney" form of infection with *B. coli*, are evident in some of the protocols.

In accordance with prevailing data and concepts, the interpretation of the intranuclear inclusions as the result of an infectious process is tempting. This theory is favored by the fact that in general the deprivation of colostrum leads to a variety of infectious diseases, and that any evidence that this deprivation causes a recognizable deficiency disease is thus far lacking. If the process is infectious it could best be assumed as due to a filtrable virus occupying the nucleus and causing the changes leading to the concretions. Moreover, like the other infec-

¹² Van Leersum, E. C., *J. Biol. Chem.*, 1928, 79, 451.

tious diseases brought to the surface by the colostrum experiments, it would have to be regarded as of low virulence, appearing only sporadically under the usual environmental conditions and perhaps very rarely in older animals. Since the subject of spontaneous renal diseases of lower animals is still in the descriptive stage, we must await further developments and the increased prevalence of this type of disease which will permit a wider, more detailed study.

Another quite different theory to account for the renal lesions is the early absorption of *B. coli* toxins from the digestive tract. Most of the animals whose protocols are given went through an early attack of liquid stools. Those that die in this stage (scours) have intensely congested kidneys. The effect of the *B. coli* toxins eliminated through the kidneys may in certain surviving cases bring about the peculiar epithelial abnormalities described. To prove or disprove this hypothesis, any more definite experimentation than the one followed (withholding colostrum, etc.) cannot be carried out because the calf after the first week cannot be infected with *B. coli* by feeding. The intravenous injection of filtrates is not a complete substitute since the toxins formed in the ileum may be either more potent or different from these in the culture tube.

Intermingled with cases of the renal disease were those in which convulsive seizures coming on suddenly and accompanied by various forced movements indicating irritation of the central nervous system were the chief symptoms. These are twitching of one ear or tail, movements of the legs, staggering gait, difficult swallowing, disturbance of vision, grating of teeth, convulsion and death within one half to several hours after the sudden beginning of the attack. The postmortem changes are hemorrhages in the intrathoracic portion of thymus, petechiae on the epicardium and large vessels, and pulmonary congestion. In 6 animals thus affected, only one (No. 1416) did not die within a few hours of the beginning of the attack. This animal became blind and lingered along for 47 days after the attack when it was killed. Various causes suggested themselves, among them botulism, status lymphaticus, acute poisoning, and anaphylactic reactions. In two of these cases intranuclear bodies were present. The rest had not been examined as thoroughly for such bodies, since the earliest cases were regarded as due to poison. Tentatively the hypothesis is put forth

that this group of symptoms may be the earlier acute fatal stage of the renal disease and that the inclusion bodies develop in the less acute type. Or, a similar intranuclear affection of epithelial cells elsewhere in the body thus far overlooked, may lead to the convulsive type.

CONCLUSION

Renal lesions chiefly the result of obstructive processes were associated with intranuclear bodies in the epithelium of straight and convoluted tubules. In one animal these bodies were found in large numbers in otherwise still normal organs. The affected animals had been fed cow serum in place of colostrum.

EXPLANATION OF PLATES

PLATE 9

FIG. 1. Frozen section of fresh kidney of Calf 1532 in physiological salt solution, showing the almost universal presence of intranuclear bodies in the straight tubules. $\times 205$.

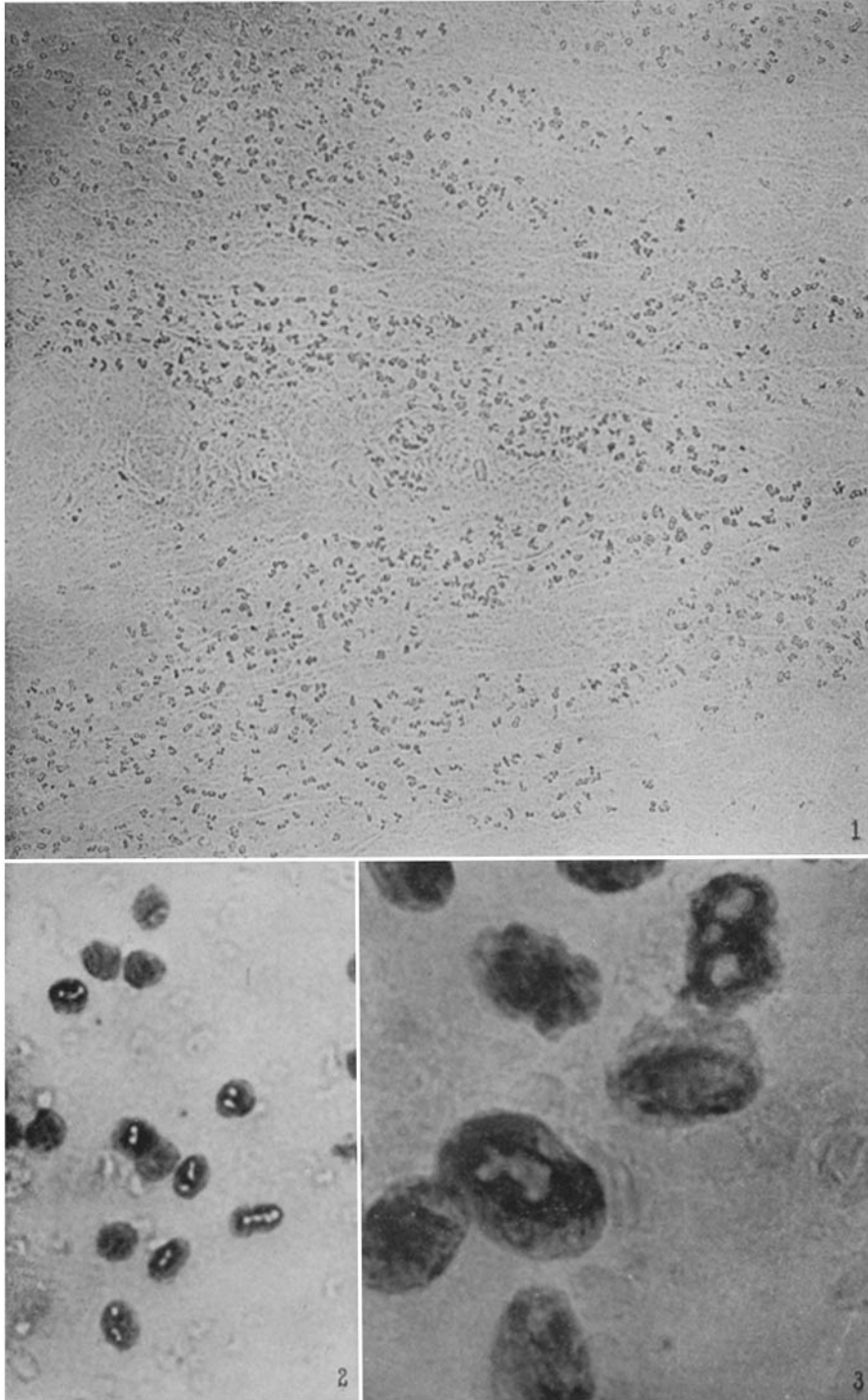
FIG. 2. Film prepared by scraping the cut surface of the fresh kidney, drying the film, passing it through absolute alcohol and water, staining in hematoxylin and eosin, clearing, and mounting in balsam. $\times 560$.

FIG. 3. The same preparation. $\times 1920$.

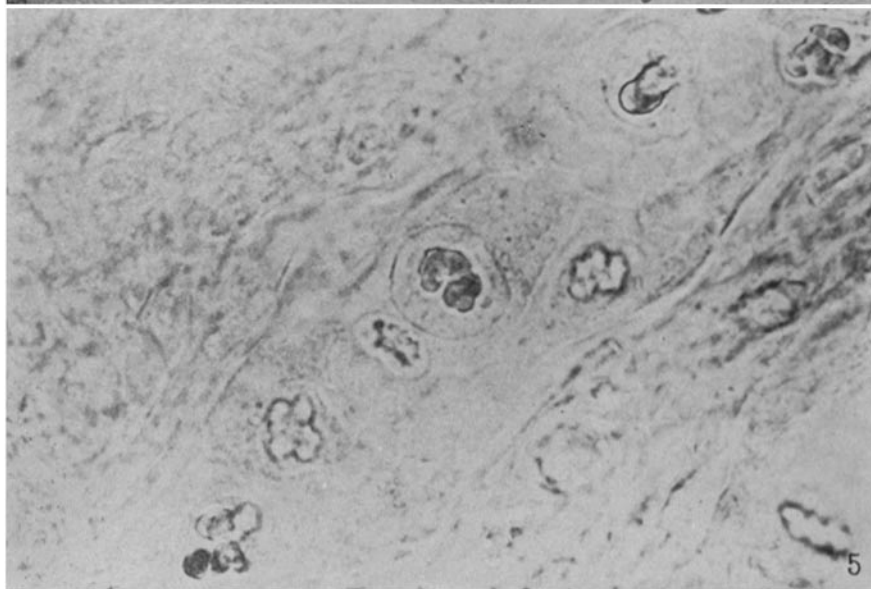
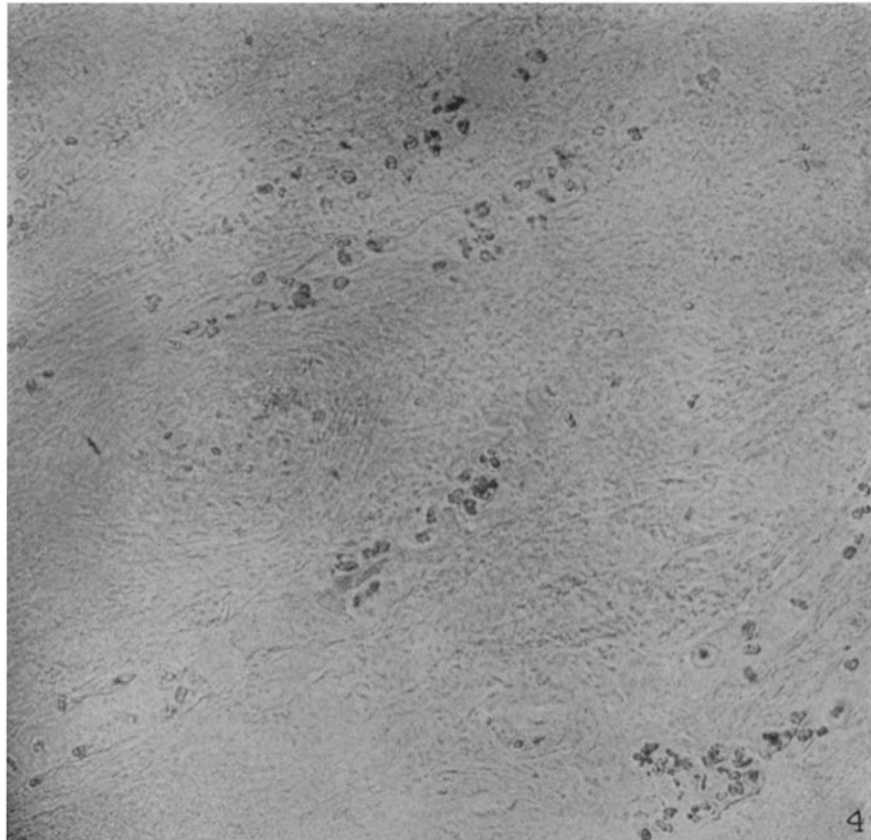
PLATE 10

FIG. 4. Frozen section of formalinized kidney of Calf 1511. Mounted in water. $\times 200$.

FIG. 5. Frozen section from the same kidney, showing the intranuclear site of the inclusion bodies. $\times 350$.



(Smith: Immunological significance of colostrum. III)



(Smith: Immunological significance of colostrum. III)