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THE FATE OF DEXTRAN IN TISSUES OF THE ACUTELY WOUNDED

A STUDY OF THE HISTOLOGIC LOCALIZATION OF DEXTRAN IN TISSUES OF KOREAN BATTLE CASUALTIES *

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Swedish workers pioneered the investigation of dextran, the polysaccharide plasma expander, in 1944 and 1945.[1] British and American studies followed. Thorsén,[2] in 1949, reported that 20,000 units of dextran had been given to 5,000 patients in Sweden. Its utilization in the United States has been restricted largely to controlled clinical and experimental studies. There now have been many reports attesting to the usefulness of dextran as a relatively short-term, blood-volume expander and to its favorable results in critical comparison with other plasma substitutes.[3-9] However, opportunities for correlative histologic studies have been rare.

Clinical Reactions. In the earlier days of dextran usage, untoward reactions, although rarely severe, were encountered frequently. These were considered referable to allergic or sensitivity mechanisms. The early Swedish dextran infusions were associated with a higher reaction rate than later products.[9,10] More highly purified dextran and a more uniform molecular size have been thought to be the important factors in reducing the incidence of reactions, so that toxic reactions are currently uncommon and of a mild degree.[7,8,11] Thorsén[12] estimated an over-all reaction rate of 0.2 per cent, with no serious reactions, calculated from 25,000 transfusion units given in Sweden. Kabat and Berg[13] demonstrated an antigenic trait of dextran by observing the development of precipitins and cutaneous sensitivity, and postulated that the occurrence of dextran in commercial sugars and its elaboration by organisms in the gastro-intestinal tract provide an explanation for systemic allergic reactions. Amspacher and Curreri,[7] in 1953, observed in reinfusion dextran studies on a large series of Army patients, some of

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whom had had initial reactions, that there were actually fewer reactions after the repeat infusions than in the initial series. None of these patients developed any demonstrable sensitivity to dextran.

More recently there has been reported a previously undescribed hemostatic defect in normal subjects after infusions of dextran.[14] This defect was characterized principally by a prolonged bleeding time. Hemorrhagic tendencies, however, were not observed in the Army-sponsored clinical trial of dextran on battle casualties in Korea.

With the gradual resolution of the side reaction problem of dextran, more attention has been directed toward its wider clinical application and the major questions have been those of its excretion, metabolism, tissue toxicity, and tissue storage.

Excretion. Dextran is known to be cleared by the kidneys fairly rapidly, but the amounts appearing in the urine of normal controls have varied considerably with the type of dextran used and the method of analysis. These values have been calculated to be in the range of 25 to 40 per cent clearance in the first 24 hours.[15] However, Lathrop and Allen,[16] by using C^{14}–labelled dextran, calculated a renal excretion of 40 to 50 per cent in the first 12 hours. Other investigators[7] have found that about 50 per cent of present-day dextran is excreted in the urine during the first 24 hours. The renal excretion then falls off so that after 72 hours it is uncommonly detected in the urine. It is also known that dextran begins to be cleared by the kidney almost immediately after the start of its infusion and the first post-infusion hours account for a marked decrease in the plasma dextran concentration. The most dramatic demonstration of dextran material in the renal tissues of experimental rats (Fig. 1) was noted in animals sacrificed 1 hour following infusion.[17] Since dextran solutions usually are composed of a rather wide range of molecular sizes, it has been reasonable to suppose that it has been the fractions of smaller molecular weight which are most rapidly excreted by the kidneys. Recently, Giebisch and Lauson[18] provided definitive data on the relationship of molecular size to renal excretion. After infusing dogs with relatively homogeneous fractions of dextran of varying average molecular weights, the average dextran/creatinine ratios of renal clearance were calculated; and the authors concluded that dextrans are excreted by a simple process of glomerular filtration without substantial tubular influence.

Granting that the majority of dextran is excreted by the kidneys, there remained the more difficult problem of accounting for approximately 20 to 40 per cent (depending largely on the average molecular weight of the infused dextran) not detected in the urine. Logically, this portion has been considered as being composed principally of the

fractions of larger molecular weight. Considerable investigation has centered about this problem and its possible relationships to metabolism and tissue storage.

Metabolism. Studies done by Lathrop and Allen[16] with C^{14}–labelled dextran indicated a recovery rate of 11 per cent of the C^{14} in the expired air (as $C^{14}O_2$) over a 4-day period. Further evidence of metabolism has been offered by an interesting experiment in which a fixed urinary dextrose/nitrogen ratio in phlorhizinized, starved dogs was elevated after the administration of radioactive dextran; and it was found that a portion of the excreted glucose, which contributed to the increased dextrose/nitrogen ratio, was radioactive.[19]

Gray and Highland[19] injected a series of mice with radioactive dextran and determined the radioactivity of total carcass fat, protein, and carbohydrate fractions. The specific radioactivity of all three tissue fractions tended to approach gradually a common value, indicating that the carbon of the glucose molecules of dextran had been incorporated into the general carbon pool of the body with a distribution in forms other than dextran. These workers concluded that the possibility of retention of dextran per se in tissues, with potential adverse effects, was no longer a valid hypothesis.

Additional proof of the ability of tissues to metabolize dextran was reported by Bloom,[20] who found that 68 per cent of dextran disappeared from the plasma of nephrectomized rats in 24 hours, as compared to 89.3 per cent for normal rats. A similar interesting observation was made in anuric battle casualties who had received dextran infusions[21] and this will be commented on later in this report.

A summarization of the experimental studies on the *in vivo* metabolism of dextran suggests that the body is capable of metabolizing the substance quite completely.

Storage. As noted previously, prior to tracing dextran in tissues with radioactive methods, there was considerable reason to suppose that the portion of infused dextran which was undetected in the urine, feces, or expired air might well have been absorbed and stored by the body. Bull *et al.*,[22] in 1949, found no chemical signs of dextran (British product) in rabbits receiving multiple injections as long as 56 days later, but did comment on serologic evidence of storage in lymph nodes and spleen. An interpretation of experiments using C^{14}–labelled dextran necessitates the consideration that the tissue assays are done by measurement of their radioactivity, which gives no assurance that the radioactive C^{14} is still bound in the intact dextran molecule. The C^{14} may well be then in a catabolite (as in $C^{14}O_2$ in expired air), or incorporated into other tissues. The more detailed experiments of Gray and

Highland,[19] previously noted, have helped to clarify such data and to de-emphasize the rôle of tissue storage as a factor of quantitative significance.

Maycock[11] has referred to minute quantities of dextran being detectable in the urine by serologic means several months after chemical analytic methods were negative. Finally, such traces of dextran were noted to disappear.

There has been experimental evidence, however, of the storage of dextran material in animal tissues. Mowry and Millican,[23] with a new technique, in 1953, reported visualization of dextran-identified material in the reticulo-endothelial cells of mice at intervals of several months following repeated infusions. Using the same method for histologic identification of dextran, I[17] have observed rather striking deposits in the spleens of rats which had received multiple dextran injections over a period of several weeks (Fig. 2).

There have been no known reports in the literature (to my knowledge) with specific reference to dextran storage in humans; however, the opportunities for such observations have been few. It is significant to mention that where dextran-identified material has been observed in the reticulo-endothelial cells of experimental animals, it has been after repeated large infusions over relatively long periods of time. More important, such foci have been unassociated with a tissue response.[17,23,24]

Histologic Manifestations of Dextran

Reported studies on the histologic manifestations and effects of dextran have been sparse. Goldenberg, Crane, and Popper[25] gave repeated infusions of a relatively unpurified grade of dextran to dogs, rabbits, and guinea-pigs. They commented on swelling and granularity of renal tubular cells with cast formation, which changes were apparently not influenced by the number of injections and which tended to reverse toward normal within 5 days following the last injection. They also described a glomerular change consisting of homogeneous, pink-staining swelling of the loops which was often severe and decreased only slightly with time following the last dextran infusion. Attempts to visualize the dextran in tissues were unsuccessful, and the authors correctly surmised that this failure to stain the substance may have been due to its being dissolved out in routine tissue preparation.

Turner and co-workers[26] in 1949, using dextran of questionable purity (10 of 30 patients had reactions of anaphylactoid type), found focal parenchymal lesions in partially exsanguinated dogs which had received dextran infusions. Reticulo-endothelial hyperplasia with the formation of giant cells was noted in the spleen. The livers of some

animals showed focal midzonal necrosis, and evidence of focal glomerular and tubular damage was seen in the kidneys. In retrospect, it seems that most of these histologic alterations were traceable to the type of dextran used, for subsequent histologic investigations have not verified a majority of these observations.

Johnston, Bennett, Lundy, and Janes,[8] in 1953, contributed interesting data on the necropsy findings in four burned patients who had received dextran (Macrodex). The only remarkable and consistent histologic change which they observed was swelling and vacuolization of the renal convoluted tubular epithelial cells. This often was associated with amorphous, eosinophilic débris within the tubular lumina. Evidence of significant pathologic findings in other organs, traceable to dextran, was absent. The renal tubular changes seemed reversible, as indicated by their being less prominent in those cases having the longest survival time following the last dextran infusion. This tended to be true, regardless of the amount administered. Johnston *et al.* referred to certain "overlapping" histologic features of the renal tubules following dextran infusions and tubular changes of lower nephron nephrosis, and they stressed the desirability of further investigation to clarify the aspects of similarity.

In this present study of necropsies on battle casualties who had received dextran, the major tubular changes referred to by both Goldenberg *et al.*[25] and Johnston *et al.*[8] were observed quite consistently. The swelling of the convoluted tubular cells, with a fine granularity and vacuolization of the cytoplasm, was identical to that of the previous descriptions and seemed to be a temporary deviation from the normal, without recognizable significance. In addition, there was opportunity to study these changes in association with a coexisting lesion of lower nephron nephrosis. No definite relationship was noted between the dextran-induced renal tubular alterations and the characteristic tubular sequences of lower nephron nephrosis. These aspects will be discussed later.

Mowry, Longley, and Millican,[24] in 1952, described a histochemical technique for the staining of dextran material in tissue preparations. In 1953, Mowry and Millican[23] observed dextran material, within 2 hours after infusions, in the blood vessels, renal tubular cells and lumina, and the liver cells of mice. The substance likewise was visible in other organs and in widely scattered phagocytes of the reticuloendothelial system after longer post-infusion intervals. Reticulo-endothelial phagocytosis was noted to persist over periods of several months following repeated infusions but in gradually diminishing amounts. Little or no dextran was seen in liver cells after 1 month. In the kid-

neys it largely disappeared from the tubular lumina in a day or so, but remained detectable in tubular epithelial cells for from 2 to 4 weeks. The authors could not determine any evidence of deleterious effects of dextran storage by reticulo-endothelial cells or any focal toxic lesions in the organs. They concluded that dextran infusions in the mouse, in almost any amount, were virtually innocuous.

Maycock,[11] in his review of plasma substitutes, cited reference to the dextran experiments of Persson[27] which showed no histologic change attributable to dextran in rabbits.

Method

In 1952, trial of dextran in battle casualties of the Korean war was approved by the Surgeon General. Satisfactory results led to limited use of dextran in Korea under the aegis of the Surgical Research Team. Liaison with the Pathology Section of the 406th Medical General Laboratory was established for histopathologic study of tissues from such patients.

The recently developed histochemical technique for localization of dextran in tissues was utilized. Mowry, Longley, and Millican[24] reported the method in 1952 and modifications were added subsequently.[23,28] The basic feature of this technique was recognition of the high aqueous solubility of dextran. By fixing tissues in absolute alcohol, processing without aqueous contact, and then staining with a modified periodic acid-Schiff (PAS) method, these workers were successful in staining dextran.

In order to gain experience with this new technique and to have histologic material as a base line for further studies on human necropsy material, a series of rats were given intravenous infusions of dextran.[17] These studies confirmed the value of the published technique and the method was adopted for analysis of necropsy tissues from battle casualties who had received dextran.

From October, 1952, until May, 1953, a special series of 15 necropsies of soldiers who had received dextran were performed in Korea. Particular efforts were made to perform the post-mortem examinations at a short interval following death. Representative tissue blocks were fixed in absolute alcohol in addition to routine fixatives. The alcohol-fixed material was processed and stained according to the modified PAS technique of Mowry and Millican.[23] Obviously this stain is not specific for dextran, for the PAS method reacts with many tissue carbohydrates and lipids. The element of confusion between these positively stained substances and dextran-related materials, however, is largely obviated by comparison with a consecutive serial control section,

stained simultaneously in the same manner, but exposed to water prior to staining. Dextran deposits, being highly soluble in aqueous solutions, are absent in the control section. The natural tissue saccharides (i.e., basement membranes, hyalin, colloid, mucin, and glycogen) are not completely soluble in either aqueous or alcoholic media and may be stained in both sections.

Some of the aspects and problems relative to the histologic identification of dextran should be mentioned. When material identified with dextran was seen in tissues, in the majority of instances it appeared as discrete purplish red granules of variable size and at times almost black. When large concentrations were present, granularity was not observed but instead solid aggregates of very dark, reddish black, homogeneous material were noted, as in the renal tubular lumina. At other times, particularly within hepatic and renal cells, the entire cytoplasm presented a rose-red stain without actual granular aggregates. Varying degrees of success were encountered in obtaining uniform staining, which, at times, defied a rational explanation, particularly when observed in identical sections of the same tissue. However, this difficulty was not of the nature of seeing "dextran-stained material" as an artefact, but rather in a tendency for dextran material to accept the stain in one area and not in another in respect to which there was no apparent reason for its not being equally demonstrable. This patchy staining affinity of dextran-containing tissues was observed likewise in the experimental rats when conditions for prompt fixation were ideal.

Observations

The following general observations were made in the histologic evaluation of dextran* in 15 necropsies of battle casualties. It should be kept in mind that this series represents a specialized group, for the average time interval from being wounded until death was about 39 hours, and the average time from the start of the last dextran infusion until death was approximately 17½ hours. This average does not include cases 5, 10, and 14 in which the infusions were given over prolonged periods. However, as noted in Table I, for 12 of the 15 patients the last infusion was in progress within 12 hours of death. These figures indicate the short temporal range of the study and suggest appropriate considerations in the interpretation of the observations.

Kidney. Dextran deposits were seen more consistently in the kidney than in any other organ studied. Likewise, dextran was seen to appear in both renal blood vessels and parenchyma with relative rapidity. In

* The average molecular weights of most of the dextran used in the clinical studies by the Surgical Research Team in Korea were 43,000 and 48,000.

TABLE I

Data upon Fifteen Casualties in Whom Dextran Was Used and the Renal Content of Dextran Graded

Case no.	Necropsy no.	Time from wound episode to death	Time from start of last dextran infusion to death	Total amount of dextran infused	Wound site	Histologic grade of dextran in kidneys	Shock status
		hrs.	*hrs.*	*cc.*			
1	J-3198	16½	6	1,000	Head	4	None
2	J-3199	32½	4½	2,000	Multiple	5	24 hours before death
3	J-3200	69½	65	1,000	Extremities	2	Entire course
4	J-3315	40½	4	1,500	Abdomen and arm	5	During last 17 hours
5	J-3426	50	47*	4,500	Burns	2	None
6	J-3430	9	6	1,500	Head	5	During last few hours
7	J-3434	6½	6	1,500	Extremities	4	Entire course
8	J-3548	6	1¼	1,500	Head and hands	1	Entire course
9	J-3694	¾	¼	500	Head	0	(Died within 1 hour of wound)
10	J-3760	17	11½*	2,000	Abdomen	3	During last few hours
11	J-3761	4½	4	500	Multiple	2	Died of hemorrhagic shock during operation
12	J-3793	106½	93½	1,500	Multiple	3	Intermittent entire course
13	J-3858	17	13	1,300	Chest	0	Terminal only
14	J-3859	42	20*	7,000	Multiple	5	During 12 hour period postoperative; none in last 20 hours
15	J-3860	167	5	1,500	Abdomen	4	Terminal 8 hours (approximately)

* Infusion given continuously until death in cases 10 and 14, and until 5 hours before death in case 5.

Histologic grading of renal dextran content:

0 = None
1 = Blood vessels only
2 = Patchy, minimal
3 = Patchy, moderate
4 = Diffuse, moderate
5 = Diffuse, marked

patients receiving large amounts within a matter of a few hours prior to death, the microscopic picture was dramatic. The entire stained section displayed a deep red mahogany color, contrasting sharply with the light pinkish red of aqueous control sections. Microscopically, the tubules contained large masses of dark positive-staining material, often completely filling the lumina (Figs. 3 and 4). These heavy aggregates were most common in the lower nephron segments and the collecting tubules. The tubular cells likewise showed staining affinity, and this was most interesting in the proximal convoluted tubules. Here, aggregates of discrete, dextran-staining granules were seen within the cytoplasm of the cells, usually unassociated with intraluminal material (Figs. 5, 6, 7, and 8). This observation likewise was made in the rat experiments (Fig. 9), corroborating the published data of Mowry and Millican[23] in mice. It is suggestive of tubular absorption or metabolism of dextran. The periglomerular spaces likewise contained granules or small clumps of the stained material.

In patients who received lesser amounts of dextran and/or at relatively long intervals prior to death, the dextran material had a tendency to be distributed in a patchy fashion and to be localized in isolated portions of the sections, principally in the convoluted tubules. This unsystematic distribution, found in other tissues as well, has seemed to be an inherent and unpredictable feature of the fixation or staining technique rather than an accurate quantitative reflection of dextran content. However, there appeared to be some degree of correlation between the amount of dextran administered and the time interval until death and the quantities seen in the sections. As noted in Table I, an attempt was made to grade the relative amounts of dextran-staining material found in the kidneys as an index for rough correlative purposes with other variables.

There was no sign of tissue damage, reaction, or inflammatory cellular response in any of the kidney sections, which could be related to the dextran. This was true also for the rat tissues, including those with multiple injections over prolonged periods.

The routine hematoxylin and eosin sections of the kidney showed the previously referred to tubular changes of cellular swelling, cytoplasmic vacuolization and granularity, and intraluminal, amorphous, eosinophilic material (Fig. 10). These findings seemed largely restricted to the convoluted portions of the nephron and, in particular, to the proximal segments. The tubular epithelial swelling occasionally was quite striking, serving to delimit sharply the convoluted tubules from the adjacent parenchyma (Fig. 11). Efforts to correlate the histologic degree of swelling of tubular cells with the quantity of

dextran administered and the involved time intervals were inconclusive on a quantitative basis. However, a rough direct relationship did exist between the amount of dextran seen in the renal tissue and the amount of tubular swelling. The fact that the tubular changes were recognized in all cases, representing a rather wide range of post-infusion intervals and dextran amounts, suggests that these alterations are produced readily and tend to disappear slowly. In none of these cases was evidence recognized of any degenerative sequences associated with this "nephrotic" picture.

Johnston and co-workers[8] commented on "overlapping" morphologic features of dextran-induced tubular changes and lower nephron nephrosis. Four of the patients in the present series had the clinical course and histopathologic picture of post-traumatic renal insufficiency (cases 2, 3, 4, and 12). The kidneys of all 4 presented the typical convoluted tubular changes which have been described, in addition to the lesion of lower nephron nephrosis. There was no particular difficulty encountered in distinguishing one lesion from the other. The major features of the so-called dextran-induced tubular changes seen in both the routine hematoxylin and eosin and Mowry PAS preparations were: (a) the sharp confinement of the lesion to the convoluted tubules (and principally the proximal portions); (b) a swollen appearance of the tubular epithelium, often with an obscuration of the precise cell borders; (c) a tendency of the cell cytoplasm to be clear and finely granular; and (d) the amorphous, eosinophilic, intraluminal material. The histologic features of lower nephron nephrosis are well known and differ from these in: (a) the location of the principal alterations (particularly distal nephron instead of proximal convoluted tubules); (b) the presence of the distinctive heme-type casts, which generally are pigmented and coarsely granular and tend to fill the lumina (in contrast to irregularly outlined islands of finely granular to wispy, strand-like masses); (c) the frequent association of tubular epithelial degenerative changes (as compared to none); and (d) the absence of impressive vacuolization and swelling of tubular cells (in comparison to these being most conspicuous in the other entity).

Interestingly enough, in the patients with associated lower nephron nephrosis, granules of dextran material occasionally were seen dispersed among the heme-cast material (Fig. 12). It is noteworthy likewise that discrete dextran-staining particles were observed within the cytoplasm of tubular lining cells, although, as in case 4, there had been a complete renal shutdown prior to a dextran infusion administered terminally (Figs. 7 and 8). These observations lead to a side issue of speculation on partial tubular function in the lower nephron syndrome.

Spleen. Dextran deposits have been described in the splenic reticuloendothelial cells of mice within short intervals following infusions.[23] In the rats used in the present investigation similar evidence was obtained, but the most definite findings were made following multiple and prolonged periods of infusion. Figure 2 is of such a spleen showing the intracellular dextran material circumferentially arranged around malpighian corpuscles. No associated tissue changes were observed. It was difficult, however, to be certain about the demonstration of minute quantities phagocytosed in the early post-infusion periods. This was likewise true for the human necropsies. On first examination, no dextran-like material was identified, but subsequent studies were suggestive of minimal focal phagocytosis; however, in no case were these deposits unequivocal. There was no recognizable evidence of tissue reaction in the human spleen.

Liver. The evaluation of dextran within the liver is complicated by the natural presence of glycogen which, being partially insoluble in both alcohol and aqueous periodic acid-Schiff preparations, appears in both slides. The dextran-staining material, however, is dissolved out in the aqueous control PAS section, and by careful comparison its intracellular and sinusoidal presence can be detected. This is often difficult, particularly when the liver cells are laden with glycogen.

In general, the human liver sections stained somewhat more intensely and diffusely with the alcoholic PAS technique than with the aqueous method. Experimentally, the hepatic concentration of dextran is more easily detected because it is possible, by fasting the animals prior to sacrificing, to remove a large portion of the confusing glycogen. Discrete phagocytosed granules of dextran-staining substance were noted also within occasional Kupffer cells of 2 cases with the longer survival periods between infusions and death. Similar localization was seen in the rat studies where it was noted most consistently in the animals receiving multiple injections over prolonged periods.

Lungs. The lungs presented a rather varied picture for dextran identification. The most common finding was that of aggregates of positive-staining granules within the blood vessels and within alveolar capillaries. There appeared to be a direct relationship between the amount of this material and the shorter time intervals between dextran infusion and death. In several of twelve cases, dextran particles occasionally were seen in the intra-alveolar spaces, sometimes intermixed with edema fluid (Figs. 13 and 14).

Pancreas. The pancreatic parenchymal cells showed no recognizable intracellular dextran-staining material. In a few cases, however, sprinklings of the positive staining granules were noted in the interstitial tissue and in peripancreatic fat.

Heart. Commonly, the myocardial capillaries were loaded with dextran granules. In only 2 cases, however, was there definite evidence of the presence of dextran granules in the stroma, and this was only patchy and light in density. The myocardial fibers, being loaded with glycogen granules, were difficult to assess without very close comparisons with the aqueous control sections. There did not, however, seem to be any localization of dextran within the muscle fibers.

Gastro-intestinal Tract. Occasional sections of stomach and intestine were submitted in the necropsy material. No dextran was identified in the walls of those organs.

Brain. Tissue blocks from the brains of 3 patients were studied. Except for the occasional sprinkling of the dextran particles within blood vessels, none was noted in the cerebral tissue.

Soft Tissues. Several of the necropsy cases and two additional surgically removed specimens included samples of striated muscle, some obtained from wound sites, and also sections of skin. Two muscle sections showed occasional foci of dextran-staining granules within the stroma. The skin sections were all interpreted as being negative save for one notable exception obtained from a severely burned patient. This will be referred to in the discussion.

Miscellaneous Organs and Tissues. Dextran-stained material was seen sporadically in the interstitial tissue of such organs as bladder, testis, and adrenal and thyroid glands (Fig. 15). As with the occasional focal deposits seen in the stroma of striated muscle, there was no predictable consistency in these findings.

Discussion

An interpretation of histologic observations in this series of 15 necropsies is limited in scope because the time intervals from dextran infusion until death were relatively short. Consequently, the possible storage of dextran in various cells or organs can be surveyed only on the basis of such changes becoming evident very early.

The tissues of these battle casualties presented some unusual considerations with reference to the normal renal clearance of dextran, for most of the patients had suffered periods of profound shock associated with extensive injuries. The Surgical Research Team at the 46th Army Surgical Hospital demonstrated, by dextran assays in the plasma and urine, the ability of these non-oliguric, severely wounded patients to begin renal clearance of dextran within an hour after the start of the infusion. Likewise, the histologic studies revealed the presence of dextran-staining material within these kidneys in the shortest available survival times.

In only 2 cases was no dextran identified in renal tissue. In one of these (case 9), the soldier received a severe gunshot wound of the head and a unit of dextran was started within minutes at the Battalion Aid Station. The patient died, however, within about 20 minutes and it is doubtful if he received much of the infusion. In the other case (no. 13), evidence of dextran in the kidneys was to be expected; failure to demonstrate it was unexplainable except possibly on the basis of a technical error in tissue preparation.

Four patients (cases 2, 3, 4, and 12) presented a particularly interesting renal study, for their kidneys showed the pathologic findings of lower nephron nephrosis. Although no previous data are available on the actual histologic demonstration of dextran in the normal human kidney, 2 of these cases (nos. 3 and 12) with renal insufficiency gave evidence suggesting a lower excretion rate. They presented the longer intervals between dextran infusions and death in the series; 65 hours and 93½ hours, respectively. Past studies on the renal clearance of dextran in normal subjects and those of the Surgical Research Team in Korea in the severely wounded without oliguria indicated that after such intervals the greater part of the substance should have been excreted. The failure of its excretion in these 2 patients may have been related to the lesion of lower nephron nephrosis. Actual granules of dextran-stained material were observed intermixed with hemoglobin casts in patients with lower nephron nephrosis (Fig. 12). An interesting corollary to these observations in cases of renal failure was the data of Howard, Frawley, Artz, and Sako[21] on dextran assays in the plasma and urine of the post-traumatic anuric soldier. These revealed a gradual disappearance of dextran from the plasma, similar to that of a non-oliguric casualty, despite renal failure. Reference was made earlier to the studies of Bloom[20] on the rat, which showed a 68 per cent disappearance of dextran from the plasma in 24 hours in nephrectomized animals. Such observations strongly support the concept of dextran metabolism, and also indicate that the kidneys probably do not play an integral rôle in the metabolic process. The visualization of dextran particles in renal tubular cells, particularly in the proximal convoluted portions, most likely indicates an absorption mechanism; and this process might be linked to the partial hydrolysis of dextran, facilitating further metabolism elsewhere in the body.

Concerning the extravascular diffusion of dextran, comment has already been made on its recognition within the interstitial spaces of some organs, where it appeared free and not within phagocytes. Two cases deserve special attention in this regard. Case 8 was a Korean private first class who received multiple penetrating wounds and was

admitted to the hospital in extremis. In addition to blood transfusions, he was given 1,500 cc. of dextran in the 1 hour and 15 minutes prior to death. Although only a minimal amount of dextran was found in the renal tissue, there were areas of marked deposition of dextran-staining granules within pulmonary alveoli (Fig. 13). The dynamics of the transference of the substance from the vascular compartment to the intra-alveolar spaces in profound shock is a matter of speculation. Similarly, whether the identified granules represent intact or altered (hydrolyzed) dextran molecules is unknown. Bollman[29] performed experiments on the extravascular diffusion of dextran from the blood in rabbits and determined that the amount of extravascular dextran was small and that, following hemorrhage, it had little influence on the mobilization of fluid available to the blood.

Another patient (case 3) was of interest in respect to the possibility of the temporary immobilization of dextran in pulmonary edema fluid. This soldier suffered multiple penetrating wounds. Despite massive blood transfusions (15,500 cc. in 6 hours), he showed a picture of shock throughout his 66-hour hospital course. He also had received 1,500 cc. of dextran in the early hours of treatment. Although there was an interval of about 64 hours from the time of dextran infusion until death, large amounts of dextran-staining granules were seen in the pulmonary air spaces. These were in association with a marked degree of pulmonary edema fluid (Fig. 14).

One of the patients (case 5) received severe burns. During his 47-hour hospital course, dextran (4,500 cc.) was administered continuously until 5 hours before death. The striking histologic finding was a heavy concentration of dextran-staining granules in the burned skin. The granules were distributed most prominently in the pools of extravasated fluid in the interstitial spaces and extended fairly deep into the corium (Fig. 16). Sections of skin of other patients failed to show dextran material. This case is of interest because of the virtual absence of dextran from all organs except the skin. The kidney revealed only minimal amounts, patchy in distribution. Renal function remained good during the patient's course; in spite of this, the dextran contained in the burned skin was not mobilized.

As previously noted, the specimens of striated muscle and skin obtained at random from the necropsy cases showed no evidence of localization of dextran in the skin, and only occasional foci within the interstitial tissue of the muscle. In addition, surgical specimens of both muscle and skin from amputation stumps were studied in 2 patients who had received dextran several hours previously. None of these tissues showed evidence of dextran localization. They were, however,

from the sites of surgical amputation and not from the actual areas of battle trauma.

The nephrosis-like tubular changes observed in kidneys following the administration of dextran were present consistently and in some cases were striking. These swollen, convoluted tubular cells presented such a distinctive picture in ordinary hematoxylin and eosin sections that their recognition was enough to warrant a strong suspicion that dextran had been administered. On consulting clinical records, this proved to be true in several cases. These tubular changes were unassociated with any cellular response or recognizable signs of local tissue reaction. As previous investigators have remarked, they appeared transient and reversible. The Mowry PAS-stained kidney sections correlated nicely with these observations; for in cases showing the most marked tubular epithelial swelling, dense concentrations of dextran-staining material were seen both intracellularly and intraluminally. Allen[30] referred to the morphologic tubular sequences incident to infusions of hypertonic sugar solutions as giving the pathologic picture of "osmotic nephrosis," a transient effect. The dextran-induced changes seem to be similar.

Concerning the reticulo-endothelial system as a storage site for dextran, the observations in this series of necropsies indicated no significant phagocytosis in either the spleen or liver. This may have been related to the short time intervals; further studies on patients with longer survival to give a greater interval between infusion and death are needed. It might be emphasized that experiments with mice[23] and rats[17] have shown definite evidence of phagocytosed dextran-staining material as long as 3 months following injection, but the relative dextran dosage in the experimental animals was high and the injections multiple. For instance, a 1 cc. dose of dextran to a 20 gm. mouse is roughly equivalent to an infusion of about 3,000 cc. for an average man. There seems little reason to expect, from the available data, that tissue storage of dextran in man will prove to be a significant factor. Even in the reported animal studies with multiple large infusions and signs of phagocytosis in the fixed reticulo-endothelial cells, no remarkable tissue changes or reactions have been recognized.[17,23]

Summary

The histologic localization of dextran in necropsy tissues of 15 battle casualties in Korea was studied.

A modified periodic acid-Schiff staining method (Mowry) was used, utilizing the aqueous solubility and relative alcohol insolubility of dextran molecules as the basic feature of the technique.

Dextran-staining material appears rapidly in the human kidney and can be demonstrated in all portions of the nephron. Intracellular granules identified as dextran have been observed in the proximal convoluted tubules of humans and of experimental rats, which suggests this mechanism as related to dextran absorption and/or metabolism.

Granules of dextran-staining substance were noted occasionally in the reticulo-endothelial cells of human liver and spleen but these were too minute and scattered for interpretive significance. However, in the rat tissues (representing multiple large infusions over long-term periods) definite and distinct focal phagocytosis was evident.

The appearance of dextran material within hepatic cells apparently occurs shortly after infusion and probably is associated with a metabolic breakdown of the dextran molecules in this organ.

There was scant evidence of the diffusibility of dextran into tissue spaces except in association with abnormal physiologic states. Specific instances of the latter were cited and included dextran immobilized in the pulmonary edema fluid of shock patients and in the skin tissues of the severely burned.

Swelling of renal convoluted tubular cells was noted to be a consistent sequel of dextran therapy. This swelling was correlated with intracellular dextran in the histochemical preparations. These changes were morphologically similar to those following hypertonic sugar infusions and appeared to be non-toxic and transient.

Four of the 15 patients had symptoms of anuria and presented the pathologic findings of lower nephron nephrosis. These were discussed from the standpoints of the effects of anuria on dextran excretion and of the significance of dextran material in the parenchyma of the anuric kidney for extended periods following infusion.

None of the tissues studied (human or experimental rat) showed any recognizable lesions referable to dextran toxicity.

The following medical officers contributed to this project by submitting necropsy tissues, clinical data, and advice: Lt. Joseph G. Strawitz, pathologist assigned to the Surgical Research Team at the 46th Army Surgical Hospital in Korea; Capt. John M. Howard, Chief of the Surgical Research Team; Lt. Col. Arthur Steer, Chief of the Pathology Section of the 406th Medical General Laboratory; Major T. R. Anderson, Assistant Chief of the Pathology Section of the 406th Medical General Laboratory; and Col. Richard P. Mason, Commanding Officer of the 406th Medical General Laboratory and the Far East Medical Research Unit.

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LEGENDS FOR FIGURES

FIG. 1. Rat M-6627. Low-power view of the medullary portion of a rat kidney 1 hour following a 1 cc. intravenous infusion of dextran, showing intense tubular concentration of the dark-staining dextran material. PAS (Mowry) stain. × 46.

FIG. 2. Rat M-6623. Survey view of the spleen of a rat which had received twice-weekly dextran infusions (1 cc.) over a 2-month period and then was sacrificed 1 month following the last injection. Phagocytosed particles of dextran-staining material are seen within the reticulo-endothelial cells about the periphery of the pale-staining malpighian corpuscles. These have a circumferential distribution. PAS (Mowry) stain. × 48.

FIG. 3. Case 2, necropsy J-3199. Kidney section of a soldier who died 32 hours after wounding. He received a total of 2,000 cc. of dextran during the terminal 10 hours of life, with the last 1,000 cc. starting 4½ hours prior to death. In addition to the many aggregates of dextran material in the tubular and glomerular space, the tubular cells show a marked staining affinity. PAS (Mowry) stain. × 120.

FIG. 4. Case 2, necropsy J-3199. Another renal section of the same case illustrated in Figure 3. The tubular intraluminal and intracellular localization of the dextran-staining substance is clearly shown. The large vein contains a dense concentration of dextran granules. PAS (Mowry) stain. × 120.

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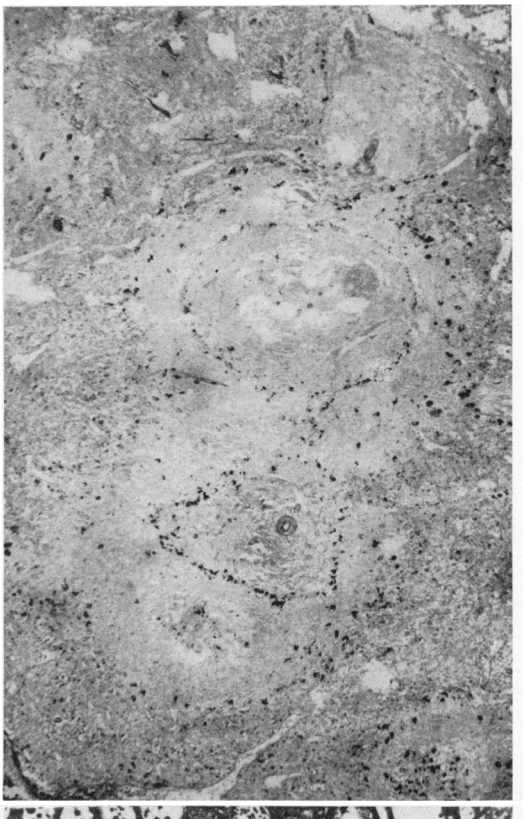

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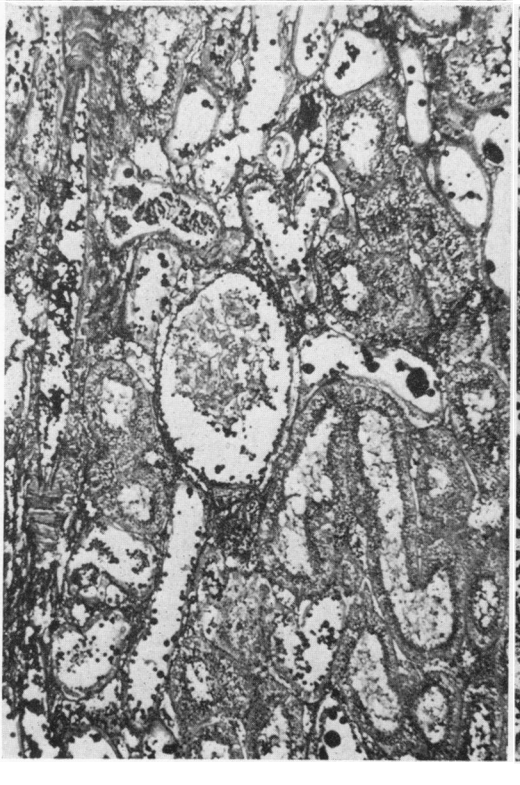

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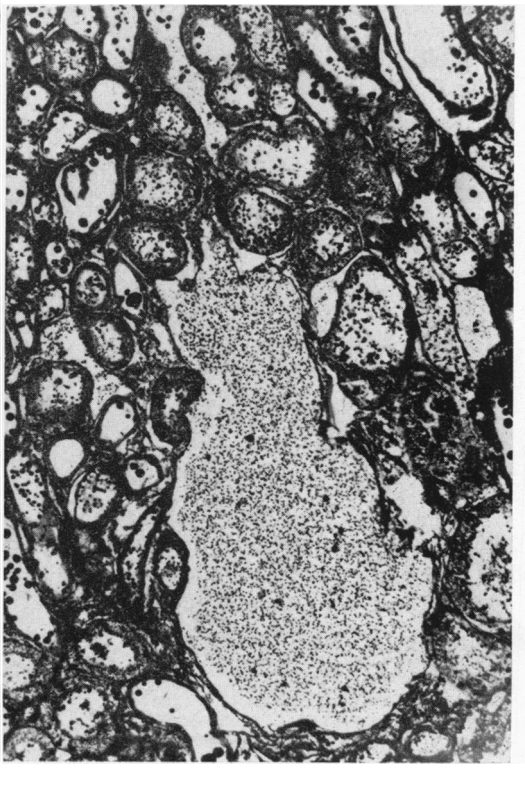

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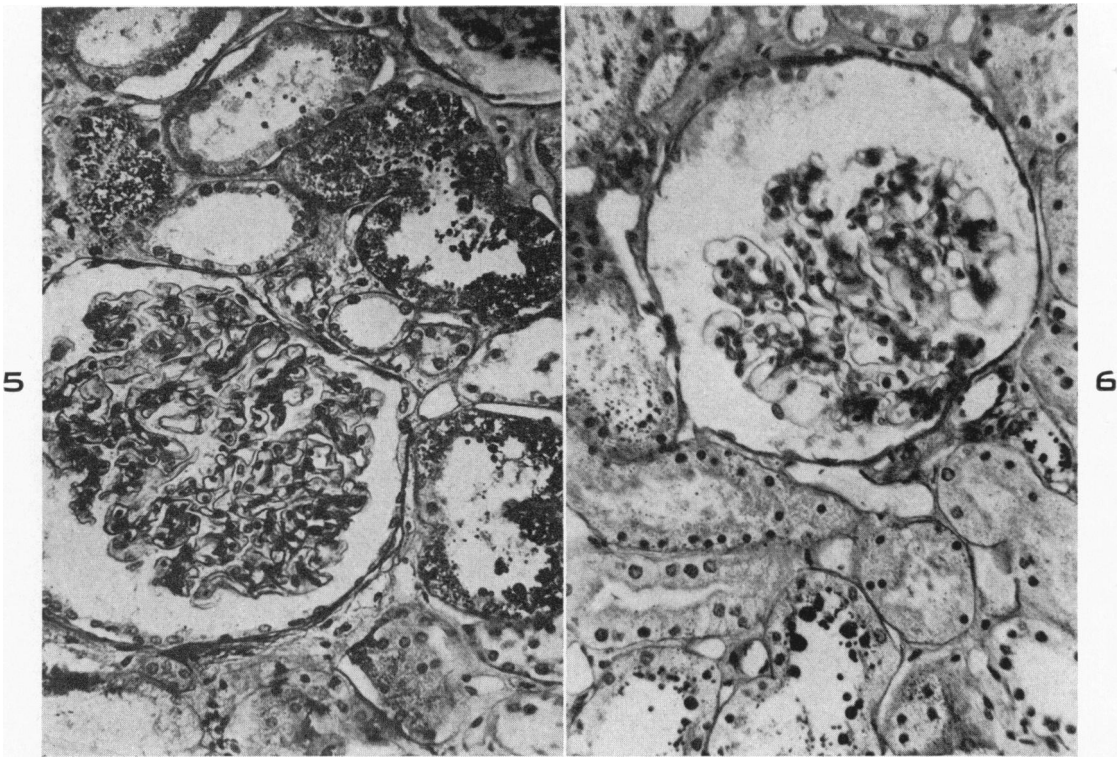


FIG. 5. Case 12, necropsy J-3793. Dextran-staining granules within the cytoplasm of convoluted tubular cells, presenting a finely stippled appearance. This battle casualty received his last dextran infusion almost 4 days prior to death. The postoperative course was complicated by oliguria (lower nephron nephrosis) and intermittent hypotension. PAS (Mowry) stain. × 240.

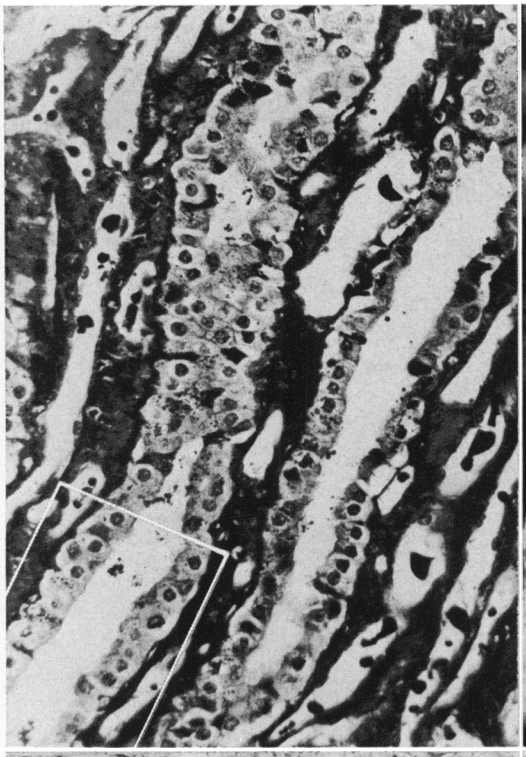
FIG. 6. Case 11, necropsy J-3761. Kidney section from a soldier who received 500 cc. of dextran at the Battalion Aid Station 30 minutes following multiple severe wounds. He died 4 hours later. Dextran-staining aggregates and granules may be noted scattered in the tubular parenchyma, both within the tubules and in the cells. PAS (Mowry) stain. × 240.

FIG. 7. Case 4, necropsy J-3315. This longitudinal view of renal tubules shows a rather diffuse sprinkling of positively staining granules within the cellular cytoplasm. The cells also appear slightly swollen. This patient received extensive wounds and developed renal insufficiency. He died 41 hours after receiving the wounds. A total of 1,500 cc. of dextran was administered: 500 cc. were given at the Battalion Aid Station and the final 1,000 cc. were infused 4 hours before death. PAS (Mowry) stain. ×240.

FIG. 8. Case 4, necropsy J-3315. This is a higher magnification of the outlined zone of Figure 7. The intracellular dextran-staining granules and the cellular swelling are illustrated. PAS (Mowry) stain. × 820.

FIG. 9. Rat 6627. Kidney section of a rat sacrificed 1 hour following a single 1 cc. intravenous infusion of dextran. (Also see Fig. 1.) The rapid appearance of the positively staining granules, not only in the distal tubular spaces, but within the proximal convoluted cells, is depicted. PAS (Mowry) stain. ×230.

FIG. 10. Case 14, necropsy J-3859. "Nephrotic" picture of renal tubular cellular swelling and vacuolization following dextran infusions. The characteristic amorphous intraluminal "casts" are evident also. This soldier suffered very severe wounds and his postoperative course, despite vigorous therapy, was marked by persistent shock. He received 7,000 cc. of dextran over the 42-hour period of survival following injury. Hematoxylin and eosin stain. × 235.



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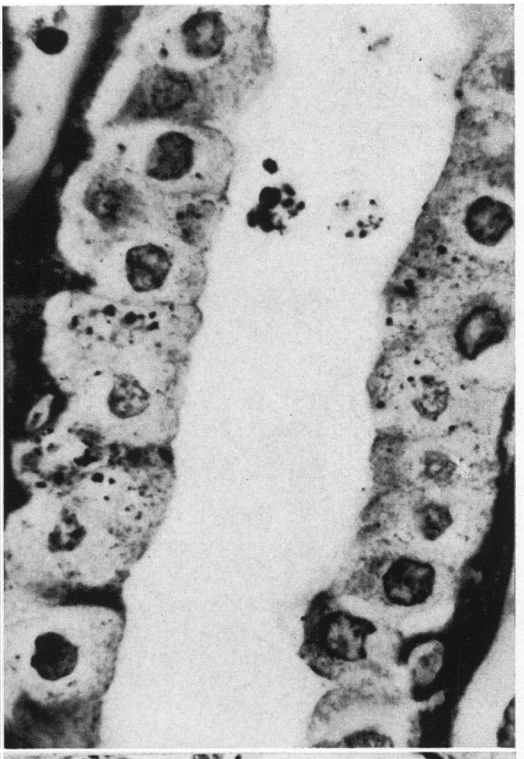

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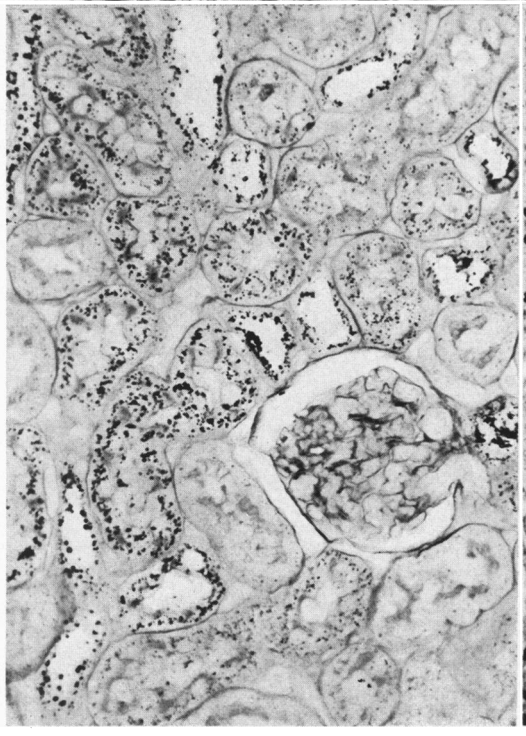

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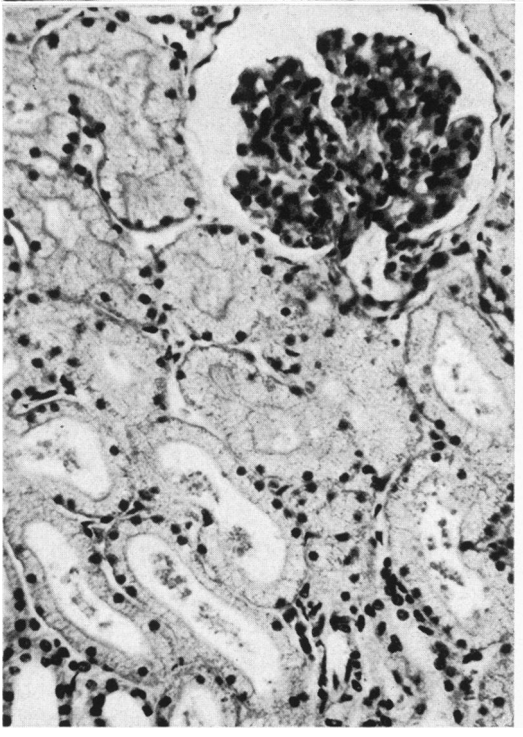

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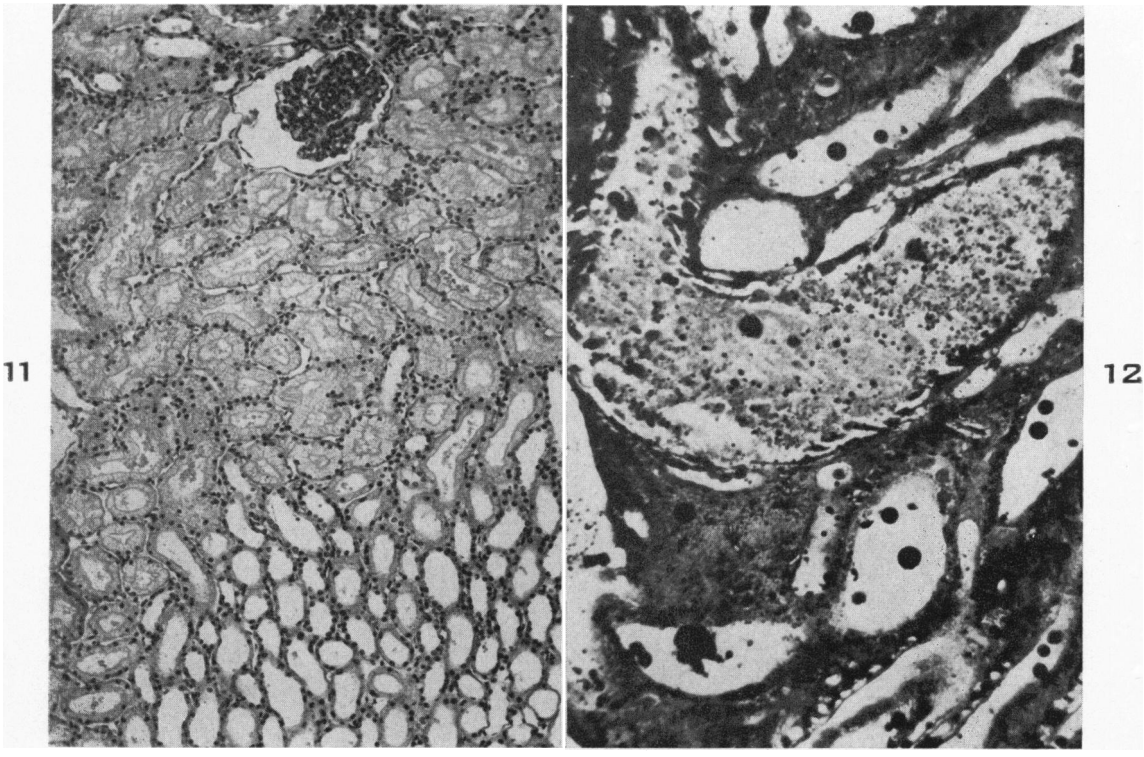

FIG. 11. Case 14, necropsy J-3859. A low-power view of the same section illustrated in Figure 10. Of note is the rather sharp confinement of the clear vacuolar swelling to the cells of the convoluted tubules. Hematoxylin and eosin stain. × 100.

FIG. 12. Case 4, necropsy J-3315. Dextran-staining aggregates and granules in renal tubules of a patient with post-traumatic renal insufficiency. Four hours prior to death, 1,000 cc. of dextran was given. The hook-shaped tubule in the center contains a typical heme cast (the lightly staining amorphous material) in which are interspersed many dextran-identified granules of irregular size. PAS (Mowry) stain. × 240.

FIG. 13. Case 8, necropsy J-3548. Dextran-staining granules within pulmonary alveolar spaces. This soldier received a severe penetrating head wound, associated with profound shock. He was given, in addition to blood, 1,500 cc. of dextran during the 1¼ hours of hospitalization before death. The presence of extravascular dextran particles in the alveoli may have resulted from the shock state. PAS (Mowry) stain. × 190.

FIG. 14. Case 3, necropsy J-3200. Dextran-staining particles intermixed with edema fluid in lung tissue of a casualty who suffered from shock throughout a 70-hour post-wound survival period. This observation is of particular interest because of the long time lapse (65 hours) from the last dextran infusion. The possibility is raised of this being a manifestation of extravascular immobilization of dextran in a patient with a protracted hypotensive course. PAS (Mowry) stain. × 240.

FIG. 15. Case 2, necropsy J-3199. A pool of dextran-staining granules in the interstitial connective tissue of the thyroid gland. (There was no local trauma to the neck.) This soldier suffered from marked postoperative shock, which probably was instrumental in causing this unusually prominent extravascular diffusion. A total of 2,000 cc. of dextran was administered during the last 10½ hours before death. PAS (Mowry) stain. × 240.

FIG. 16. Case 5, necropsy J-3426. This is a section of severely burned skin, which injury occurred following explosion of a stove. In addition to blood, 4,500 cc. of dextran were given over most of the 2-day survival period. Dispersion of fine, dextran-staining granules in the edematous upper corium may be noted. The other organs were either negative for any dextran-identified material or showed only small foci. PAS (Mowry) stain. × 230.

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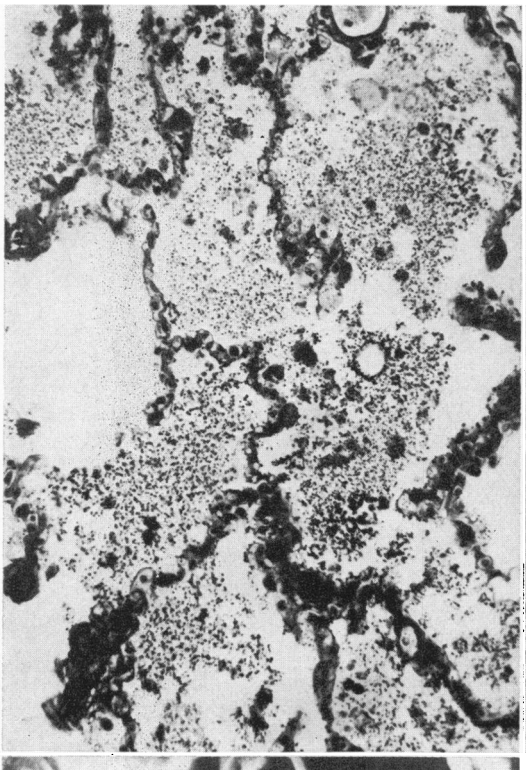

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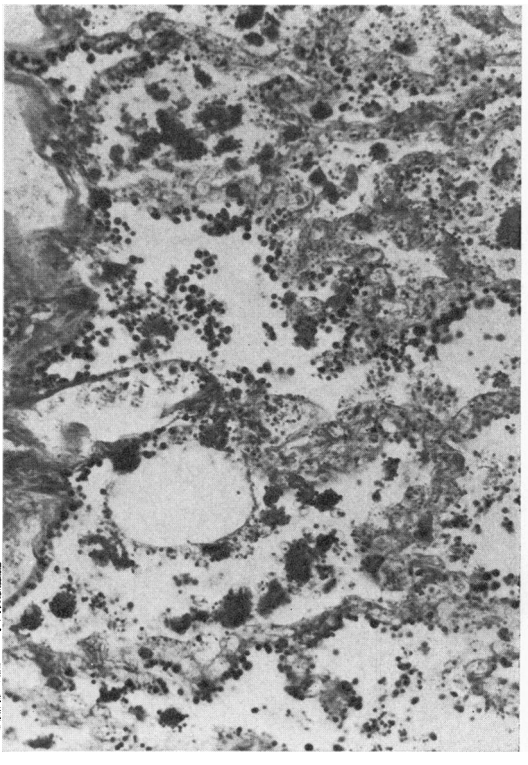

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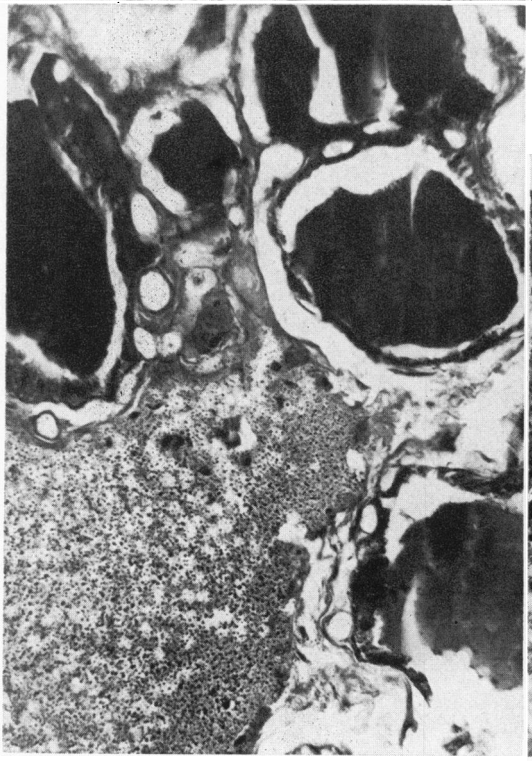

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