MYOCARDIAL NECROSIS IN THE POTASSIUM-DEPLETED RAT: A REASSESSMENT

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IN 1937, Schrader, Prickett and Salmon reported that myocardial necrosis occurred in rats placed on a potassium-deficient diet. They found patchy, complete destruction of muscle, more in the left than the right ventricle. The valvular tissue was seldom involved, the auricles never. The necrotic muscle was replaced by scar tissue and numerous phagocytic cells.

In reports published since then, there has been considerable difference of opinion as to the histological features and localisation of the lesions. Thomas, Mylon and Winternitz (1940) noted that lesions were most marked subendocardially, that there was no involvement of vessels, that the cellular exudate was predominantly macrophage in type and that deficiency of B-complex vitamins aggravated the lesions. This last point was refuted by Darrow and Miller (1942) who found that thiamine and pyridoxine supplements had no protective effect in potassium depletion. Follis, Orent-Keiles and McCollum (1942) reported that while lesions were frequently subendocardial, they never involved the endocardium itself. Kornberg and Endicott (1945) stated that auricular lesions did occur. Lesions produced by high dosage of desoxycorticosterone acetate, and described as " rheumatic fever " by Selye and Pentz (1943) were shown by Peschel, Black-Schaffer and Schlayer (1951) to be due to potassium deficiency. These latter authors noted the presence of numerous Anitschkow cells, and confirmed the occurrence of auricular lesions. French (1952) was the first to point out that the necrosis of potassium deficiency differed from that of ischaemia or cauterisation in that the lesions remained vascularised throughout, and showed no destruction of the connective tissue framework.

Similar lesions have been described in the hearts of mice (Liebow, McFarland and Tennant, 1941), pigs (Thomas *et al.*, 1940), cats but not dogs (Darrow and Miller, 1942) and humans (Goodof and MacBryde, 1944 ; Rodriguez, Wolfe and Bergstrom, 1950 ; Perkins, Petersen and Riley, 1950 ; Luft, Ringertz and Sjögren, 1951). McAllen (1955) was the first to suggest that massive myocardial scarring in the human might result from potassium deficiency.

During a personal study of other aspects of potassium depletion in rats, a cardiac lesion was regularly found which did not seem to correspond with the available descriptions. Because of this, an attempt was made to correlate the histological findings in the heart of the potassium-deficient rat with changes in the electrocardiogram and tissue chemistry. Only the histological findings are presented here. In all, 69 potassium-deficient rat hearts were studied, after varying periods on the potassium depletion regimen. Some animals were repleted with potassium before being sacrificed, to observe the effect on the lesions.

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MATERIALS AND METHODS

Adult male albino rats averaging 180 g. in weight were placed on a prepared casein-dextrin-arachis oil diet with added vitamins, as described by Cooke, Segar, Cheek, Coville and Darrow (1952). Duplicate analysis showed that this diet contained 5 mg. potassium per 100 g. dry feed. Average consumption with liberal feeding was just under 40 g. per kg. rat per day, so that each animal received considerably less than 1 mg. of potassium per day. The only drinking fluid was a mixture of 0·25 per cent $NaHCO_3$ and 0·25 per cent NaCl in water. Each animal was given 2 mg. DOCA intraperitoneally daily for the first week. Sodium sulphonic resin in the sodium cycle (" Resonium A ", Bayer), 2·5 g. per 100 g. dry feed, was mixed with the diet each day, giving approximately 1 g. resin per kg. rat per day ; this is comparable to human dosage levels.

Marked and rapid potassium depletion was produced by this regimen, spontaneous deaths occurring from the 20th day onwards. Animals were sacrificed by ether anaesthesia and exsanguination from the inferior vena cava. This provided blood for analysis and relatively blood-free organs for histological study. As a routine, a 2 mm. cross-section was cut from the ventricles mid-way between apex and base. This was fixed in Helly's fluid, the rest of the heart being placed in 15 per cent formol-saline-calcium for further study. After embedding in paraffin wax, sections were cut at 5μ and stained with haematoxylin-eosin, Mallory's azo-carmine, Mallory's phosphotungstic acid-haematoxylin, Best's carmine, periodic acid Schiff, Sudan III and IV and Gordon and Sweet's modification of Wilder's reticulin method.

RESULTS

While there are great variations in the appearance of the lesions at various stages of potassium depletion, all can be related to three basic processes. These are :

1. Degeneration and death of muscle fibres.
2. Phagocytosis of necrotic material.
3. Collapse of the supporting stroma.

Important negative features are the absence of capillary ingrowth, though not of capillary reaction, absence of a fibroblastic response and virtual absence of polymorphonuclear leucocytes.

EXPLANATION OF PLATES

FIG. 1.—Hypokalaemic necrosis. An early lesion, showing varying degrees of loss of striations, but no cellular infiltration. Seventh day of depletion. Mallory's phosphotungstic acid haematoxylin. × 250.

FIG. 2.—A small lesion showing cellular infiltration. Arrows point to some of the prominent capillary nuclei. (See also Fig. 4.) Twelfth day of depletion. Haematoxylin and eosin. × 265.

FIG. 3.—A large subendocardial lesion in an example of " hypokalaemic necrosis." (Also see Fig. 5.) Twentieth day of depletion. Haematoxylin and eosin. × 220.

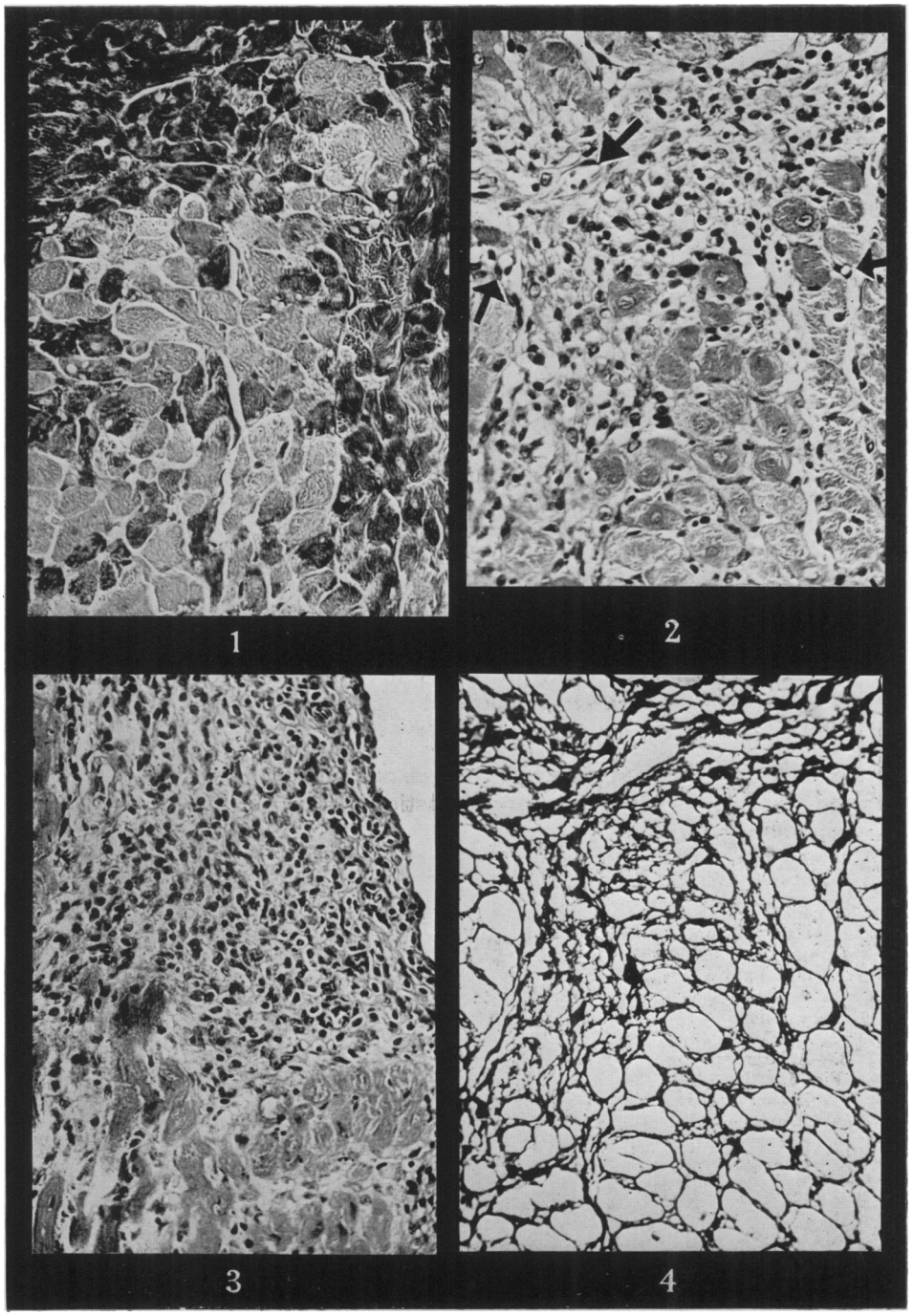
FIG. 4.—Reticulin pattern of the lesion shown in Fig. 2. Note there is compression and collapse, but little distortion of the pattern. Wilder's reticulin method. × 250.

FIG. 5.—Reticulin pattern of the lesion, Fig. 3. There is irregular collapse and compression, but little destruction of the pattern. Wilder's reticulin method. × 400.

FIG. 6.—A severely affected heart showing marked separation of the muscle bundles. Arrows point to grey necrotic foci where phagocytosis is almost complete. Dark areas are foci of more recent necrosis. The black in the upper left-hand corner is artefact. There is very little normal myocardium. Twenty-fifth day of depletion. Azo-carmine. × 16.

FIG. 7.—Reticulin pattern in a lesion of at least 6 weeks' duration. The circular structures in the lesion are capillaries. The faintly-staining lines show the staining reactions of collagen, the dark lines those of reticulin. Sixty-three days after the start of depletion, 42 days after repletion. Wilder's reticulin method. × 300.

FIG. 8.—Accumulation of glycogen in muscle fibres bordering a necrotic focus. Arrows point to some of the larger deposits. Twenty-second day of depletion. Best's carmine. × 480.



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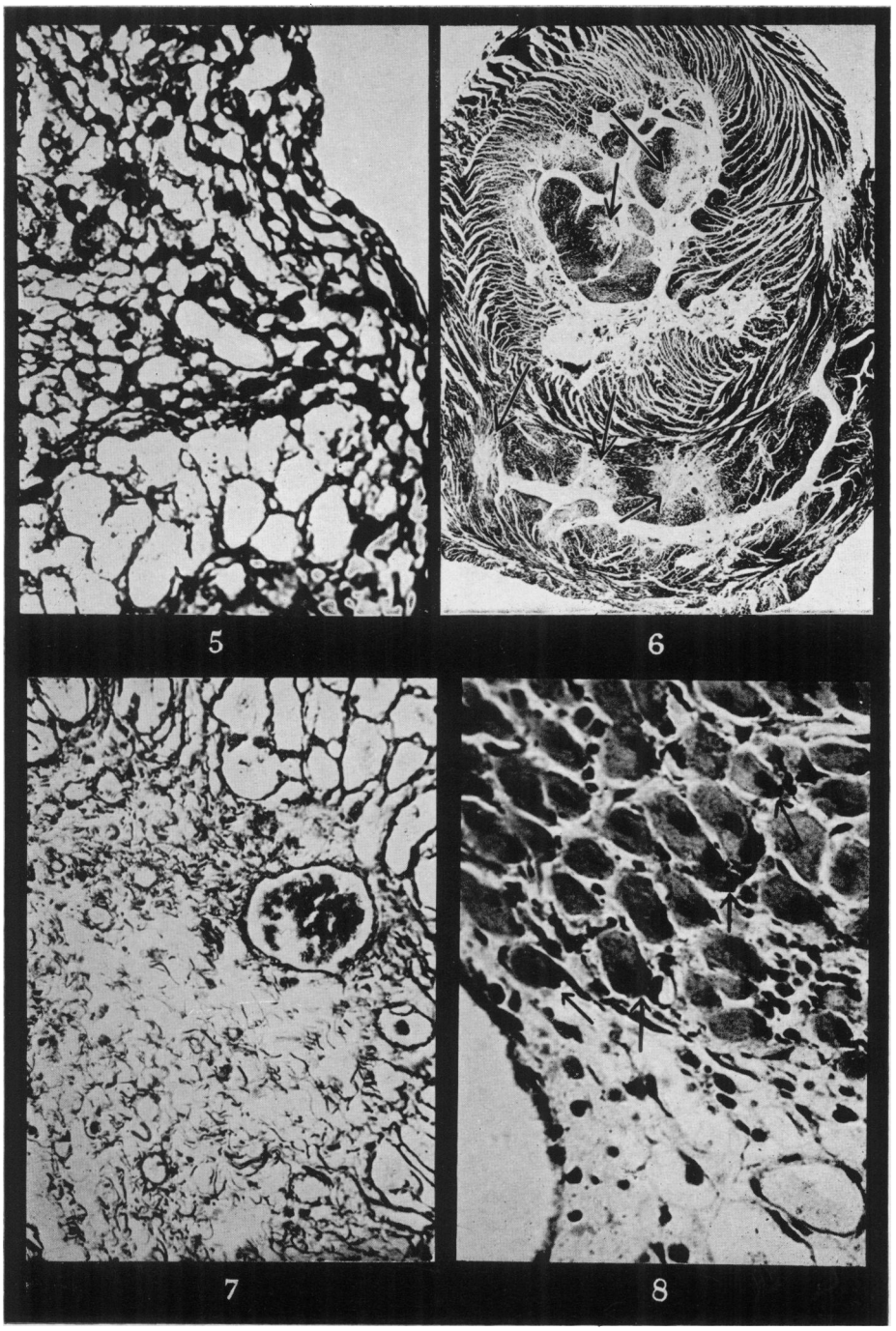

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The various stages of the lesion, and the sequence in which they occur, will be described, and then the analogy to other commoner lesions will be traced. It should be emphasised that fresh lesions continue to appear throughout potassium depletion so that all stages may be seen in a heart examined after, say, 20 days of depletion. Also, since serial biopsy is impossible, any estimate of the duration of the various stages can be only approximate.

The earliest stage of the lesion, at the end of the first week of depletion, can be detected only by special stains. It consists of some swelling of the muscle fibres and gradual loss of striations (Fig. 1). Following this the nucleus usually undergoes karyolysis, fading and disappearing. Pyknosis is also seen but its frequency is more difficult to estimate, since the pyknotic nuclei of inflammatory cells can be seen in degenerate muscle fibres from an early stage. Some of the neighbouring fibres show clear vacuoles, as described in McAllen's Case 1 (1955). These vacuoles do not contain fat or glycogen.

Soon after (by the 8th day of depletion), or even simultaneously with this loss of striations, cells accumulate round the affected fibres ; this is the first stage which can be detected by routine haematoxylin-eosin staining (Fig. 2). The cells consist mainly of tissue macrophages, but sarcolemma nuclei can also be seen, and an occasional polymorph. The cells have been described as being predominantly polymorph in type, but on close examination this is obviously not so. Even in the largest and acutest lesions (" hypokalaemic myocarditis ", Rodriguez *et al.*, 1950) the same type of inflammatory cell is seen (Fig. 3). What appears in these cases to be a widespread polymorph infiltration of the rest of the myocardium, can be seen on closer examination to consist of darkly-staining tissue macrophage nuclei, very prominent capillary endothelial nuclei and only an occasional polymorph or eosinophil. These tissue macrophages have also been described as " Anitschkow myocytes " (Peschel *et al.*, 1951), but while their derivation may be the same, the typical Anitschkow nuclear pattern is seen in only a small proportion.

In every report the presence of fibroblasts has been noted. Many of the nuclei appear to resemble fibroblastic nuclei, although the more closely they are examined the more of them are found not to have the typical appearance of fibroblasts. Moreover, as the lesion progresses, there is no evidence at all that they lay down collagen, nor are they transformed into fibrocytes. It is felt that the term " fibroblast " should be reserved for cells which have the function as well as the appearance of fibroblasts.

A most important feature, first noted by French (1952), is that there is no destruction of the connective tissue framework, and this is best demonstrated by reticulin impregnation. There is usually some collapse of the reticulin pattern, even in the early stages, but there is no destruction. This is shown by Fig. 4 and 5, which are the reticulin preparations of the areas shown in Fig. 2 and 3. Collapse of the stroma usually begins while phagocytosis is still active.

When the phagocytic cells disappear (11th day onwards), collapse becomes complete in the smaller lesions and they can no longer be detected by routine staining but azo-carmine shows such a lesion as a bright blue line with thinner lines radiating from each end, the whole sandwiched between bright red surviving muscle fibres. In larger lesions, collapse is delayed, or is only partial, resulting in a network of open spaces with almost no cells. However, a few normal capillaries always traverse such areas. This can be seen from about the 15th day of depletion.

Particularly in relation to large lesions, oedematous separation of the muscle bundles may be very striking (Fig. 6). The degree of separation varies with the acuteness of the lesion and also to some extent with its situation, as those near the endocardium or epicardium more often show it than those in the depth of the muscle. In the most severe cases, of which Fig. 6 is an example, the separation may be generalised.

Another feature which is also more prominent in the larger lesions is the presence of small irregular muscle fibres with prominent closely-set striations, near the edge, and sometimes in the centre, of the collapsed stroma. The appearance suggests that these are possibly regenerating fibres. Apart from this the lesion remains indolent and largely acellular. There is no evidence of capillary budding or ingrowth of new vessels, nor are fibroblast-like cells seen at this stage, which has been found to persist for up to 10 weeks. Gradual disintegration of the reticulin occurs, the strands taking on the staining reactions of collagen. Sometimes reticulin and collagen may be seen in the same fibre, in a way strongly suggesting that reticulin is being converted to collagen rather than that collagen is being laid down from outside the area.

Fig. 7 shows the reticulin preparation of such a lesion, which is at least 6 weeks old, the animal having been given potassium after 3 weeks of depletion to prevent the occurrence of further fresh lesions. The appearance is quite unlike that seen 6 weeks after ischaemic necrosis.

It seems that the variation in the time required for the lesion to disappear—4 days to at least 10 weeks—depends principally on the size of the lesion. However, no matter how long it persists, a dense scar is never formed. Indeed, examination of hearts up to 4 months after correction of the potassium depletion suggests that all these lesions will ultimately disappear, given sufficient time, but it would be difficult to prove this conclusively.

Since the hypokalaemic lesion differs in aetiology, pathogenesis, healing and end-result from the post-necrotic or post-inflammatory scar, it is felt that confusion might be avoided to some extent by not using the term " scar " at all for the former lesion.

A striking feature of the hypokalaemic lesion is the accumulation of glycogen in damaged muscle fibres. This is not peculiar to hypokalaemia as it can be seen in relation to foci of ischaemic necrosis, but it is unusually widespread in severe potassium depletion. It rapidly clears on giving potassium, except in the cells bordering necrotic foci, where it may persist for weeks (Fig. 8). This glycogen stains very poorly with Schiff's reagent, but it is glycogen, since it is removed by prior treatment with diastase. In the macrophages found in the lesions, on the other hand, material is found which stains red with Schiff's reagent and with Best's carmine, and this is not all glycogen, since only some of it is removed by diastase. The suggestion that this represents the removal of excess ground-substance (French, 1952) seems logical. The amount of glycogen found in the muscle-fibres varies greatly, from a few granules to a mass filling half the cell, but it is always in excess of the amount to be seen in normal cardiac muscle. In view of the known metabolic effects of potassium, this probably represents an accumulation of glycogen which the cell is unable to metabolise, but why it should persist in apparently normal cells which happen to be near a necrotic focus is not evident.

The position as regards accumulation of fat is very different. Deposits of fat were infrequent, never gross, and bore no relationship to the necrotic foci.

Although a specific search was made, no lesion could be found in skeletal muscle, which could be attributed, unequivocally, to potassium deficiency.

DISCUSSION

The present study confirms that lesions occur in the auricles, though they are small and unimpressive. Lesions are seen throughout the ventricles, but the largest are subendocardial, especially in the papillary muscles and trabeculae. This would seem to indicate either that local strain and trauma determine the localisation of the necrosis, or more probably that they aggravate the lesion once it is established. Variation in local blood supply, however, cannot be excluded as a factor. Large lesions are less common subepicardially and distinctly uncommon in the depth of the muscle.

The statements of Follis *et al.* (1942) that the lesions never involve the endocardial surface, and that thrombi are not seen, were confirmed. The way in which intact endocardium covers large areas of completely necrotic muscle is striking. As French (1952) observed, such endothelium is much more cuboidal and crowded together than is usual in the endocardium. If the mechanism involved in this necrosis of muscle is considered, it is not surprising that the endothelium should survive.

It has been shown that the supporting reticulin network remains intact, and the same holds for the endothelial basement membrane. Since connective tissue has a very low potassium content, almost identical with that of extra-cellular fluid, there is no real reason why it should be sensitive to potassium lack. The nutritional requirements of the endothelium of endocardium and capillaries are probably of the same order, and survival of normal capillaries in the middle of necrotic foci suggests that their potassium requirements are not great.

On the basis of the alkaline phosphatase reaction of the capillaries, French (1952) concluded that there was definite evidence of capillary ingrowth into the necrotic foci. However, in the present study no unequivocal capillary budding or ingrowth was seen, but abnormally prominent capillary endothelial nuclei were seen throughout the myocardium in the more acute lesions, and it was considered that the appearance of the capillaries in and around necrotic foci was due to hyperplasia of pre-existing vessels, not to newly-formed vessels.

The conflict between Thomas *et al.* (1940) and Darrow and Miller (1942) as to the value of B-complex vitamins in protecting the myocardium against the effects of potassium deficiency, was not specifically investigated. However, the use of double the amount of B-complex vitamin supplement recommended by Cooke *et al.* (1952) did appear to reduce both the number and extent of the lesions.

The Analogy between Hypokalaemic Necrosis and Other Lesions

As potassium depletion progresses, there is impairment of heart-muscle function. This is shown by changes in the electrocardiogram (low voltage, disappearance of T-waves and spreading of the QRS complex) and by the intracellular accumulation of glycogen. Why this proceeds in places to necrosis is not evident, although local trauma may play a part. For reasons already discussed the connective tissue and blood vessels are not visibly damaged and the reaction to the necrotic muscle is low grade and purely cellular. For this reason, if muscle

regeneration were sufficiently active, " *restitutio ad integrum* " would be theoretically possible. As it is not, the lesion can heal only by collapse and condensation of the connective tissue framework, with eventual reabsorption as a long-term sequel. Potassium repletion accelerates repair and prevents the development of fresh lesions but has no other obvious effect.

An analogous process is seen in mild cases of infective hepatitis. Here also the connective tissue framework is intact, the reaction is cellular rather than vascular and mononuclear rather than polymorph. However, because of the great regenerative powers of the liver cells, restoration of the normal architecture is complete, but this is not possible when the connective tissue framework is also damaged (Dible, McMichael and Sherlock, 1943). Similarly, Oliver, MacDowell and Tracy (1951) stated that in acute tubular necrosis complete restoration of normal architecture and function could occur only if the basement membrane remained intact. By contrast, in toxic necrosis of muscle (*e.g.*, "Zenker's necrosis"), even if the sarcolemma sheath is intact, " *restitutio ad integrum* " occurs only in very small areas ; this is due to the limited regenerative capacity of the muscle cell (Wright, 1950).

It must be emphasised that while potassium deficiency in the rat appears always to produce an identical cardiac lesion, varying only in degree, it is by no means the only process which can produce such a lesion. A very similar picture in the human heart is caused by diphtheria toxin (Warthin, 1924) and it also closely resembles the necrosis caused in the rat heart by mercury and uranium poisoning (unpublished observations). The ischaemic lesion in the human heart, called " myocytolysis " by Schlesinger and Reiner (1955) is also similar.

Reports indicate that potassium depletion produces an essentially similar cardiac lesion in humans, mice, rats, cats and pigs, but not dogs. For this reason, one is reluctant to accept that potassium depletion caused the dense scar found in the second case described by McAllen (1955). Additional atypical features in this case were involvement of the endocardium, mural thrombosis and embolisation, and " only slight concentric diffuse fibrous endarteritis in the larger vessels ". Since the patient had a classical attack of myocardial infarction two years before death, with two subsequent recurrences, ischaemia seems to be a much more probable cause of the scarring than electrolyte disturbance. The presence of relatively normal blood vessels in random sections of the left ventricle does not exclude the possibility of a localised vascular lesion. However, the other scattered areas of " fibrosis " in the left and right ventricles appear to be typical hypokalaemic lesions.

SUMMARY

Cardiac necrosis is found in the potassium-depleted rat from the end of the first week onwards. There is oedematous separation of muscle bundles and accumulation of glycogen in damaged muscle fibres, but connective tissue and blood-vessels are not involved. Inflammatory cells consist almost entirely of macrophages. Healing is by collapse and condensation of the stroma and is complete in the vast majority of cases. Dense scars never result. An analogy is drawn between this lesion and other commoner conditions.

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