TABLE I. Experimental Data of 3 Centrifugation Runs of Mixtures of Haemocyanine and Yellow Fever Virus Strain 17D. $a = 26°$. $X_1 = 5.32$ cm. $S_1 = 100$ Svedberg units.

| r.p.m. (×100) | $H_1$, cm | $H_2$, cm | $LD_{50}$ of samples taken at cm: 0.5 | 1.5 | 2.5 | 3.25 | 3.5 | 3.75 | 4.25 | 4.5 | 4.75 | BL | Original, 6 cm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 180 | 2.0 | 4.0 | 0 | 0 | 0–1 | 2.4 | — | 3.0 | 3.8 | — | 4.0 | — | 4.0 |
| 198 | 3.1 | 5.25 | 0–1 | 0–1 | 0–1 | — | 1.8 | — | — | 3.5 | — | — | 4.5 |
| 244 | 4.75 | B | 1 | .6 | 1.0 | — | 1.5 | — | — | 1.5 | — | 4.5 | 4.9 |

B = bottom; BL = bottom layer.

where r is the radius of the particle, S the sedimentation constant, $\eta$ the viscosity of water at 20°C, d the density of the virus particle assumed to be 1.33 g/cc and $\rho$ the density of water at 20°C. The diameter of the particle determined in this way ranges from 29.2 to 31.4 m$\mu$.

*Discussion.* The size of yellow fever virus particle as determined in this work is nearer that found by Bauer and Hughes (l.c.) in ultrafiltration experiments than that recorded by Pickles and Bauer (l.c.).

*Summary.* The particle size of the 17 D strain of yellow fever virus has been determined as ranging from 29.2 to 31.4 m$\mu$ by the method of ultracentrifugation coupled with biological assay.

The author wishes to express his gratitude to Professor M. van den Ende for his interest in this work, and to Miss T. Madsen for technical assistance. The virus used in this work was very generously supplied by Dr. J. H. S. Gear of the Poliomyelitis Research Foundation, Johannesburg.

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## Influence of Alpha-Tocopherol upon Development of Cardiovascular Necrosis and Hypertension in the Rat.* (20970)

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Medical literature is replete with contradictory reports on therapeutic efficacy of vit. E. Thus large dosages of alpha-tocopherol or mixed tocopherols have been reported to exert therapeutic effects in muscular dystrophies and rheumatic diseases of men(1-3). Also that prolonged administration of this vitamin may bring about dramatic amelioration in hypertensive cardiovascular disease, as well as many peripheral vascular conditions(4-6). However, well controlled clinical studies by others failed to confirm the reported benefits(7-10). The vast animal experimental literature dealing with vit. E contains surprisingly few references to the beneficial action of this vitamin, if one takes exception to specific effects in induced deficiency. With regard to the cardiovascular system, only 2 publications described therapeutically desirable effects. Allardyce and associates(11) reported a hypotensive effect of vit. E in desoxycorticosterone induced hypertension of albino rats, and Holman(12) was able with the help of vit. E to inhibit emergence of experimental necrotizing ar-

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teritis induced in dogs on a "standard fat" diet by way of renal injury.

In view of these latter reports and the controversial nature of clinical observations, it seemed of interest to evaluate the influence of vit. E upon development of hypertension and cardiovascular necrosis induced in the albino rat with the help of renal injury, using a short-term method developed in our laboratory(13).

*Materials and methods.* Closely bred 10-months-old albino rats of both sexes from our own colony were employed. Animals were maintained on Purina Chow. For the actual tests, subgroups of at least 5 rats each were placed on a special complete synthetic diet (14). Since cardiovascular necrosis and hypertension were produced by procedure previously described(13), only the main features of this method will be briefly restated. A single excessive dose of sodium acetyl sulfathiazole is injected intraperitoneally into adult albino rats. This induces severe "obstructive nephropathy" due to massive deposition of poorly soluble sulfonamide crystals in the renal tubules. Impairment of the kidney in turn results almost invariably in the development of extensive disseminated necrosis in the arterial tree as well as the myocardium, and frequently also, in the muscularis of the stomach within a period of 5 to 7 days. Necrosis is followed by a stage of reparative changes with scar formation and intense calcification. Marked hypertension developing about 5 days after renal block, lasting for 2 to 5 weeks, is a usual feature of the intoxication. It was calculated that control animals eating standard synthetic diet received about 0.8 mg of alpha-tocopherol daily. Experimental diet was enriched to yield a daily intake of 10 mg alpha-tocopherol† per rat. After 2 weeks, rats eating the enriched food received supplementary stomach tube feedings of alpha-tocopherol in olive oil (10 mg/100 g body weight), 3 times weekly for 4 weeks. This was to ensure an uninterrupted intake of alpha-tocopherol in excess, since spontaneous food consumption drops sharply after production of renal injury. Rats eating the normal synthetic diet received intubations of equal volumes of olive oil. Kidney damage was induced in both groups after third intubation. A total of 33 rats were employed. Of these, 2 groups of 5 animals each, served as normal diet and vit. E diet controls, respectively. They were not exposed to renal injury and were killed after completing the full course of alpha-tocopherol or olive oil intubation on the 29th day. In the experimental group exposed to renal injury and subsequent cardiovascular damage, 13 rats (8 ♂ and 5 ♀) were kept on alpha-tocopherol enriched regimen, whereas 10 rats (5 of each sex) were fed the control synthetic diet.

Food intake was determined daily and the body weight checked twice weekly. Systolic blood pressure was recorded at predetermined intervals with the help of a photoelectric tensometer which measures volume changes in the hind leg of unanesthetized rat(15). All measurements were made by one experienced experimenter. Except for an occasional moribund animal which was not subjected to the recording procedure, all control and experimental rats were measured at the same session. The mean of at least 3 to 5 consecutive satisfactory readings at intervals of several minutes was taken to represent the systolic blood pressure. The animals were killed at predetermined intervals by exsanguination in ether anesthesia. Blood was used for estimation of N.P.N., calcium, and inorganic phosphates. All rats, including those dying spontaneously as a consequence of renal injury, were subjected to complete postmortem examination. The heart, thymus gland, adrenals, and kidneys were weighed immediately after removal on a torsion balance to the nearest mg. These organs as well as the aorta and gastrointestinal tract, were examined under the microscope using routine and special stains.

*Results.* Data on the incidence and severity of the most important organic lesions found in experimental animals, after exposure to renal injury, are depicted in Table I and Fig. 1.

A glance at the life span of individual ani-

† Liberal supplies of alpha-tocopherol acetate were obtained through the courtesy of Dr. A. Pirk of Hoffmann-La Roche, Inc., Nutley, N. J.

TABLE I. Production of Disseminated Muscular Necrosis in the Albino Rat. Influence of excessive amounts of alpha-tocopherol.

| Days killed or died* after renal block | Heart, necrosis, calcification, cell infiltr. Myocard. | Coron. vessels | Aorta, medio-necrosis, perivasc. infiltration | Stomach, necrosis and calcification, muscularis | Kidney, pyeloneph. calcification, nephrosis |
|---|---|---|---|---|---|
| Alpha-tocopherol enriched synthetic diet | | | | | |
| Males | | | | | |
| 6* | 3+ | 3+ | 3+ | + | 4+ |
| 8* | 2+ | + | 4+ | 3+ | 4+ |
| 9* | 3+ | 3+ | 4+ | — | 3+ |
| 13* | 3+ | 2+ | 4+ | 0 | 4+ |
| 16* | 2+ | 3+ | 4+ | 0 | 2+ |
| 29 | — | 3+ | 2+ | ± | 3+ |
| 29 | — | 2+ | 4+ | 2+ | 3+ |
| 29 | — | 2+ | 4+ | 3+ | 3+ |
| Females | | | | | |
| 7* | 2+ | 3+ | 4+ | 0 | 3+ |
| 13* | 2+ | | 4+ | 0 | 3+ |
| 23 | + | 2+ | 4+ | + | 3+ |
| 23* | 3+ | 3+ | 4+ | — | 4+ |
| 50 | ± | ± | 2+ | — | 3+ |
| Normal synthetic diet | | | | | |
| Males | | | | | |
| 7* | 3+ | + | ± | — | 2+ |
| 7* | ± | — | — | — | 2+ |
| 8* | 2+ | + | ± | — | 2+ |
| 62 | — | | 3+ | — | 3+ |
| 62 | — | + | — | — | 3+ |
| Females | | | | | |
| 7* | 3+ | 2+ | 3+ | 0 | 4+ |
| 13* | 4+ | 2+ | 3+ | 0 | 4+ |
| 62 | — | + | + | — | + |
| 63 | — | 2+ | 4+ | + | 3+ |
| 50 | + | 2+ | 3+ | — | 4+ |

— = Normal organ (macroscopically and microscopically).
± = Definite but minor damage, recognizable under microscope.
+ = Considerable damage, recognizable under microscope.
2+ = Marked change, often visible to naked eye.
3+ = Advanced change, easily recognized upon gross inspection (except coronary vessels).
4+ = Severest degree of abnormality.
0 = Organ not examined.

mals (Table I) demonstrates that the extent of vascular, gastric or renal changes is not noticeably altered by variations in time of survival from 6 to >60 days following renal block. Myocardial damage, on the other hand, is most conspicuous within the first 2 weeks and shows a tendency towards complete healing in animals surviving 4 weeks or longer. Accordingly all 14 rats that died within the first 23 days, showed myocardial damage, usually of marked degree, whereas of the remaining 9 animals surviving more than 4 weeks only 2 manifested minor changes in the heart muscle. In view of the fact that most of these latter animals still had severe renal and vascular damage, it was assumed that they probably also had myocardial changes at an earlier date. This is supported by our findings in other recent studies with groups of rats killed within 14 days after renal block. As in the corresponding group of the present study, the incidence of typical myocardial alterations was usually close to 100%. It is obvious, therefore, that in comparative evaluation of changes in the heart, time elapsing between renal injury and death is of major significance.

With regard to changes in the muscularis

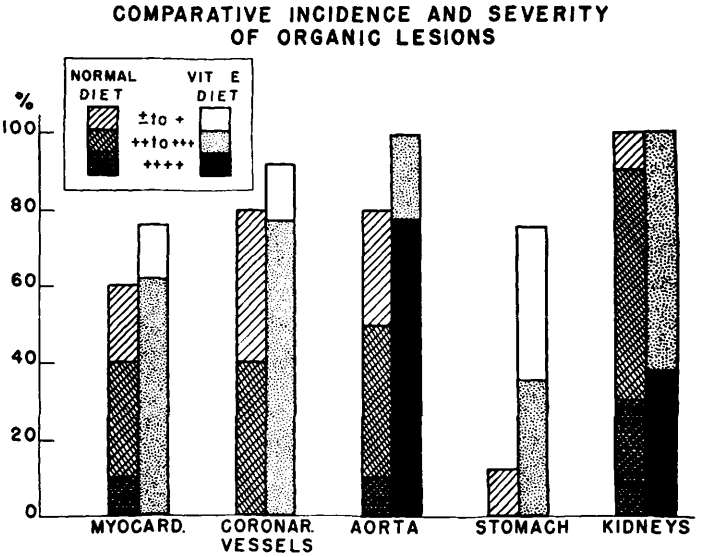


FIG. 1. Note substantial difference in *severity* of organic changes in coronary vessels, aorta and stomach of albino rats. (For explanation of + and — symbols see Table I.)

of the stomach it should be pointed out that advanced autolysis made it impossible to examine the stomachs of 6 rats. Of these, 2 were on control diet and 4 on vit. E enriched regimen. Nevertheless, the absence of any damage in the stomach wall of 7 out of 8 rats eating the normal diet, and the presence of necrosis in the muscularis of 6 out of 9 stomachs examined, from the vit. E group including 3 with advanced necrosis and calcification, adds considerable weight to the impression that damage of smooth muscles is enhanced in the presence of alpha-tocopherol in large amounts. This becomes particularly noticeable when the pathologic-anatomical alterations are grouped in accordance with the degree of damage (Fig. 1). Although, except for the stomach, total incidence of organic lesions is apparently not materially greater in rats receiving large dosages of alpha-tocopherol than in animals maintained on a normal diet, the distribution of coronary and especially aortic lesions according to extent of damage is highly suggestive of an aggravating effect of alpha-tocopherol.

Of the 5 control rats maintained on the alpha-tocopherol enriched regimen without being exposed to renal injury, 2 animals showed small foci of nephritis as the sole organic change. This insignificant lesion is not infrequently observed as a spontaneous change in normal rats.

No substantial deviations from the norm were found in the blood calcium and inorganic phosphate levels of experimental animals, as determined in samples obtained at sacrifice, which meant at least 3 to 4 weeks after date of renal injury. The N.P.N. levels, on the other hand, were still elevated in many instances and remained above the normal range even after 50 and 60 days.

Following production of renal block, the rats of both diet groups suffered usual loss of body weight, about 20% after one week and more than 40% in 3 weeks. Thereafter, with increasing appetite, body weight usually returned to its initial value at about the fifth week. Control animals on both diets showed expected maintenance or slight gain in body weight.

Finally, the effect of alpha-tocopherol upon the systolic blood pressure of normotensive and hypertensive albino rats is depicted in form of a scatter-gram (Fig. 2). The figure contains individual blood pressure records of all 23 experimental rats on 5th and 3rd day before production of renal block and of survivors of this group on the 8th, 16th, and 22nd day after renal injury. In addition, this graph also contains blood pressure read-

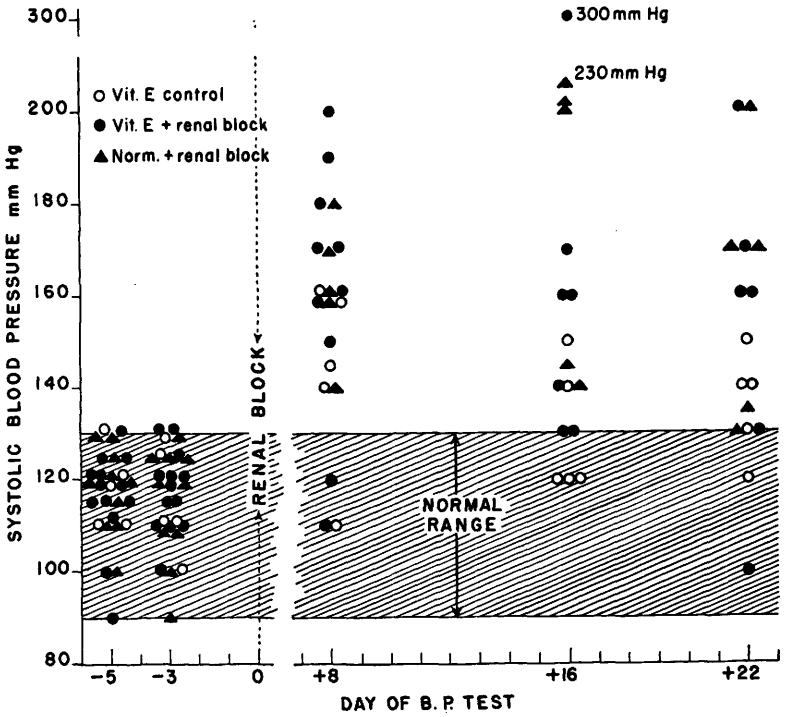


FIG. 2. Individual systolic blood pressures of 28 albino rats. In 23 of these rats a renal block was induced at zero day. The other 5 animals served as vit. E diet controls. Note marked hypertension resulting from renal injury and moderate elevation of systolic blood pressure caused by alpha-tocopherol administration.

ings of 5 control animals exposed to vit. E diet regimen. It is apparent that definite though moderately hypertensive levels were registered in some of the control rats on several occasions under the influence of alpha-tocopherol intubation (white circles). In contrast the blood pressure of 5 controls intubated with olive oil only, remained within normal range. (For the sake of greater clarity the blood pressure values of these latter controls were not included in the graph.)

Renal block, on the other hand, induced expected pronounced elevation of systolic blood pressure in the majority of experimental animals. No substantial difference in incidence or degree of hypertension could be derived from this small group of survivors feeding on either of the 2 diets. It is quite clear, therefore, that presence of abundant amounts of alpha-tocopherol in the body of the albino rat, does not noticeably interfere with the development of renal hypertension (black circles).

During the 14-day period of control observations before production of renal damage, systolic blood pressures of all experimental and control rats had kept well within normal range of 90 to 130 mm Hg.

Characteristic pathological anatomical changes in the cardiovascular system, gastrointestinal tract, adrenals, thymus gland, and kidneys were closely similar upon gross inspection and under the microscope to those observed in previous studies(14); also the change in moist weight of the 3 last named organs. The macroscopic and microscopic appearance of vascular lesions is illustrated in Fig. 3. Typical histological changes in the kidneys, the myocardium and the muscularis of the stomach are shown in Fig. 4. Lesions produced in presence of alpha-tocopherol in excess appeared to differ in degree of intensity rather than in quality from the organic damage induced in rats on a normal diet.

*Comments.* This study demonstrated that under the conditions of our experiments,

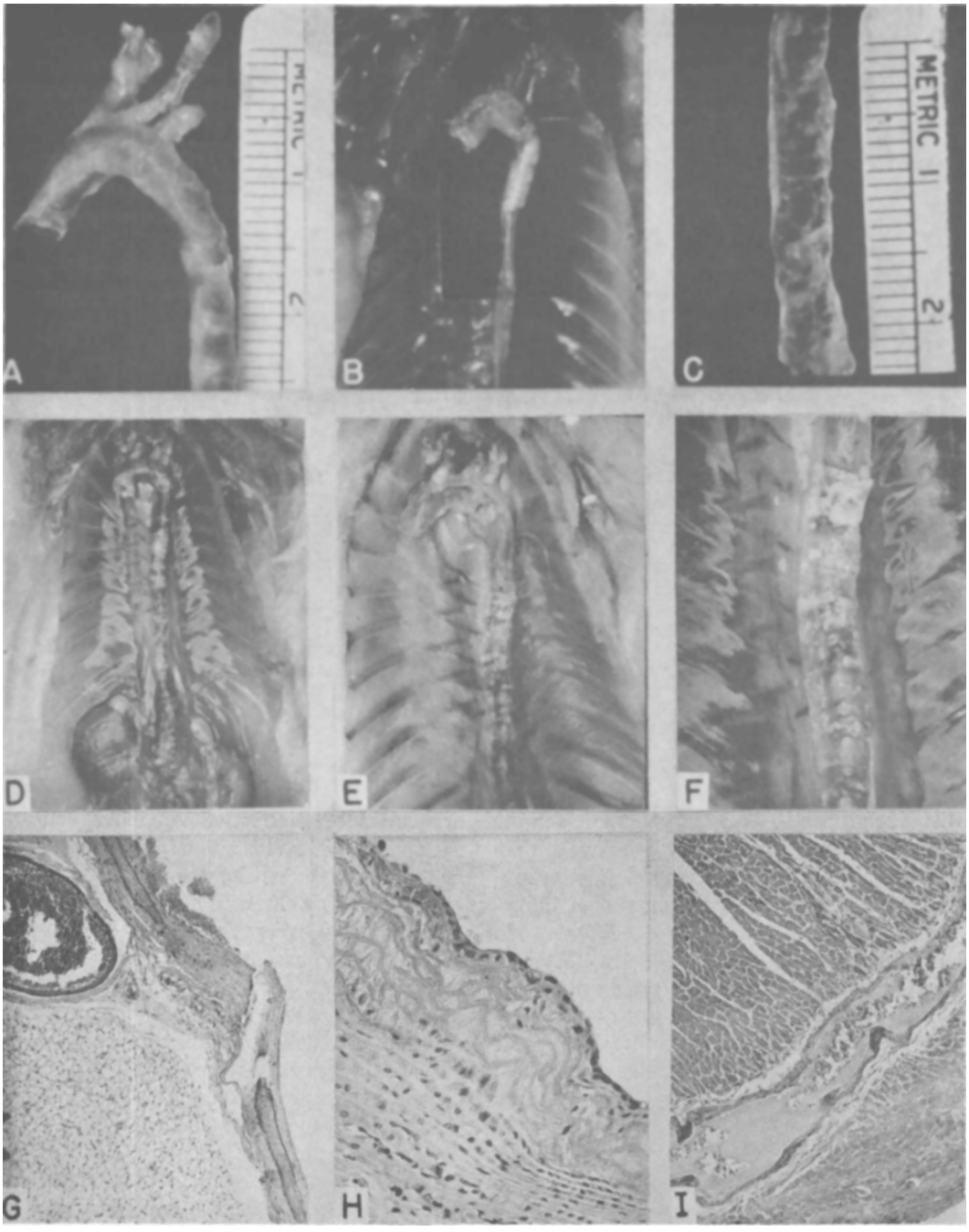


FIG. 3. Typical macroscopic and microscopic appearance of arterial lesions in alpha-tocopherol fed albino rats following renal injury. (Animals 1-13, Table I.)

A. Aortic arch and large branches. Bamboo-stick lesion of aorta extending into innominate, carotid and subclavian arteries.

B. Calcified aneurysm of aortic arch and upper part of descending aorta. Note normal thin-walled aorta below crossing of V. azygos. (Protection apparently due to mechanical pressure of vein.)

C. Abdominal aorta. Multiple aneurysmic dilatations of thinned out wall.

D. Dilation and bamboo-stick calcification of arch and descending aorta. Also visible are enlarged adrenal glands and contracted kidneys.

E. Open view of the aorta of Fig. D.

F. Same as Fig. E, close-up. Note irregular, bulging sub-intimal nodules. There is superficial resemblance to human atheromathosis.

G. Aorta and a large branch. (Left upper corner of photograph) showing necrosis and calcification of media.

H. Wall of aorta. Subintimal necrosis of media with swelling and breakdown of elastic membranes, and proliferation of endothelial cells over the lesion.

I. Longitudinal section of coronary artery. Wall is markedly thinned out, necrotic and irregularly calcified; lumen is filled with coagulated plasma and red cells.

alpha-tocopherol had no ameliorating influence upon the development of hypertension nor upon cardiovascular necrosis induced in the albino rat by way of renal injury. On the contrary, the presence of abundant supplies of this vitamin appeared to enhance muscular necrosis in the wall of blood vessels and stomach of these animals. In addition, alpha-tocopherol was found to be capable of producing moderate elevation of systolic blood pressure of normotensive control rats. These observations are at variance with the results of 2 experimental studies previously mentioned. Thus Allardyce and associates(11) reported that during daily administration of vit. E in 10 mg dosages (route of administration not stated), systolic blood pressure of rats, apparently stabilized at 300 mm Hg due to administration of desoxycorticosterone, showed a gradual decline to 250 mm Hg. However, only 6 rats were employed. Since only 3 of these animals received vitamin alone, whereas the remaining 3 were given histidine concomitantly, the significance of the observation is open to doubt. Our contrary finding of a blood pressure raising effect of vit. E is in line with the warning of Shute(16) that large dosages of alpha-tocopherol (300 mg or more daily) may cause a very considerable rise in both the systolic and the diastolic blood pressure of patients with hypertensive heart disease. It is also in conformity with the experimental data of Telford and associates (17) who reported about 30% reduction of systolic blood pressure in vit. E deficient rats as compared to normal control animals. Holman(12) who saw a preventive effect of vit. E in experimental arteritis induced in 8 dogs by "standard fat diet" and "standard renal damage," suggested that arterial lesions are induced by piling up of "toxic" substances (fatty acids) which are prevented from spilling over into urine when the safety valve of the kidney is destroyed.

The vascular lesions which we observed in albino rats were similar though far more severe than those seen by Holman. And yet, they developed in the absence of cholesterol and all animal fats from the diet of this species. Moreover, in experimental atherosclerosis caused by cholesterol feeding, vit. E was reported to have either no beneficial action(18) or to be responsible for accentuation of the characteristic arterial changes(19-21).

In trying to explain beneficial or detrimental effects of tocopherols, one becomes keenly aware that, among vitamins, vit. E in the physiology of the body is least well understood. It has been suggested, on the basis of knowledge of the effects of vit. E deficiency in the rat, that a major role of the vitamin *in vivo* is the control or restraint of one or more oxidative reactions, either by limiting the rate of some normally occurring reactions or by preventing the occurrence of detrimental reactions that take place only in its absence (22). It is well known that deprivation of this vitamin is followed by a bewildering array of functional and organic disturbances in different species of animals and that many of these disturbances can be cured in a striking manner by alpha-tocopherol administration (23). There should, of course, be no reason to assume that presence of large amounts of a substance must necessarily have a beneficial action upon tissues which suffer damage due to its depletion, and yet, judging from clinical reports, much of the reasoning in therapeutic application of vit. E seems to have proceeded in this manner.

In view of the many contradictory claims regarding the therapeutic capabilities of vit. E at the bedside, it would seem desirable that a more determined effort be made to distin-

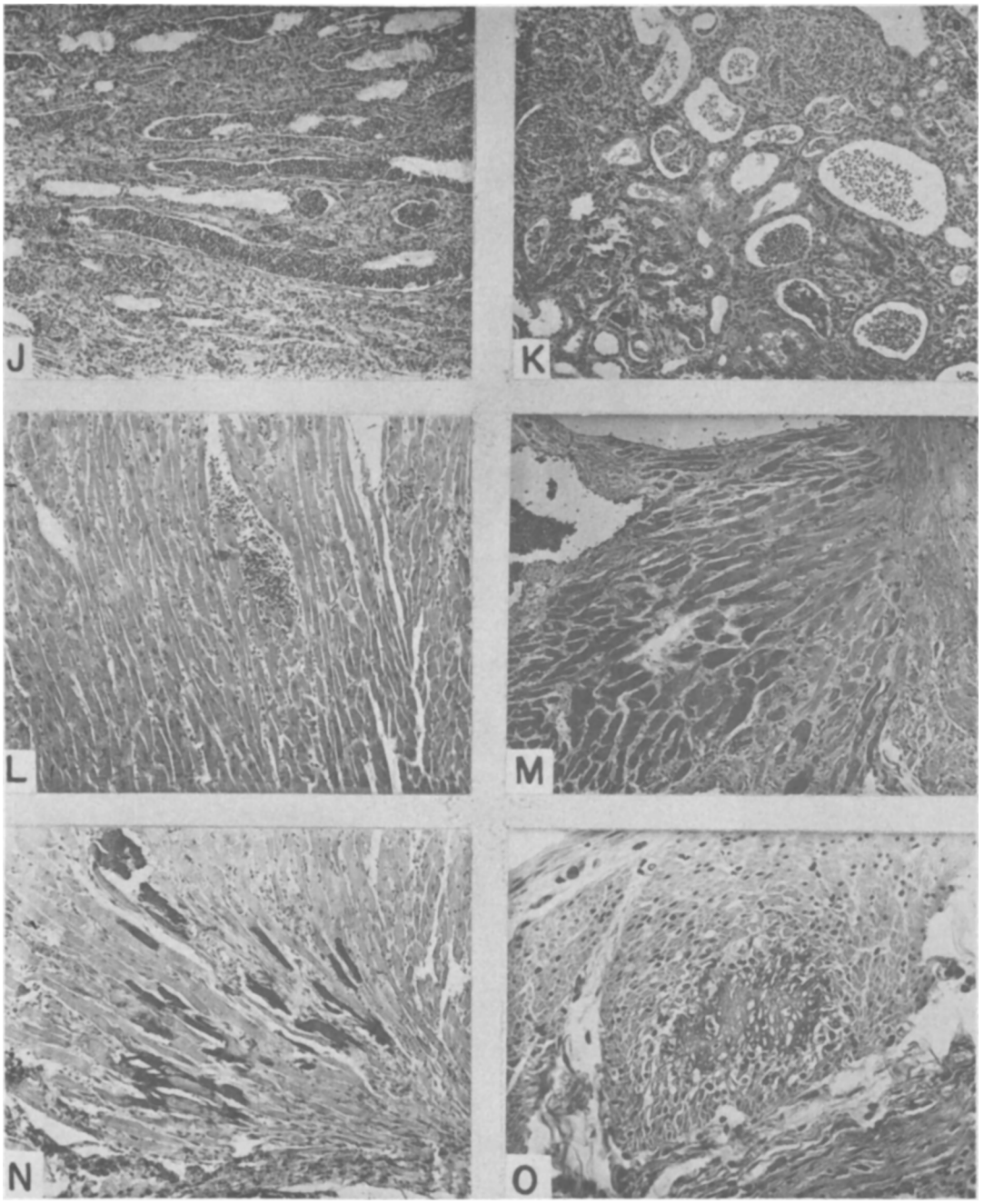


FIG. 4. Typical histological appearance of changes in kidney, myocardium and muscularis of stomach in alpha-tocopherol fed albino rats following renal injury. (Animals 1-13 of Table I.)

J. Ascending pyelonephritis in renal medulla. Note large tubular casts composed of polymorphonuclear leucocytes and interstitial infiltration.

K. Typical lesion of renal cortex. Showing tubular dilatation with casts of pus cells, interstitial inflammation, necrosis and calcification of tubular epithelium and foci of tubular regeneration.

L. Interstitial myocarditis. Diffuse infiltration and focal collection of predominantly mononuclear cells.

M. Focus of myocardial necrosis. Note darkly stained necrotic fibers in left lower corner of photograph.

N. Myocardium. Necrosis and calcification of individual muscle fibers.

O. Stomach wall. Showing necrosis and calcification in muscularis.

guish specific substitution effects in deficiency from pharmacodynamic effects obtained with relatively large dosages in the nondeficient organism. Most clinical applications of vit. E obviously belong to the latter group. In this connection it may be of interest to quote the chief proponents of vit. E therapy in cardiovascular disease(24): "Vit. E therapy in our hands is not substitution therapy but a form of chemotherapy. This is a point that deserves emphasis. The doses we use are larger than nutritional studies would demand, but usually smaller dosage is ineffectual. We think of alpha-tocopherol as a chemical compound which also happens to be a food constituent, but whose dosage level in established cardiac disease is not closely related to that coincidence. . . . A small dosage of E gives but little hint of what it can accomplish in massive dosage." We are in agreement and believe that our experimental studies have tested the drug effect of alpha-tocopherol in massive dosage, rather than a specific vitamin action.

In search for an explanation of the detrimental drug effect of alpha-tocopherol we were intrigued by the view of Lecoq *et al.*(25), who ascribe alkalizing properties to vit. E. They demonstrated that a permanent increase in nerve chronaxia due to hypervitaminosis E could be brought back to normal by acidification with the help of ammonium chloride. Since it was demonstrated by one of us(26) that ammonium chloride is capable of inhibiting and even preventing emergence of cardiovascular necrosis in the albino rat, we are now investigating the possibility that an alkalizing action of the vitamin might have been responsible for its deleterious influence in our experiments.

*Summary.* 1. Disseminated muscular necrosis following experimental nephropathy in albino rats maintained on complete rations, was not beneficially influenced by the presence of excessive amounts of alpha-tocopherol. On the contrary, this drug appeared responsible for a higher incidence of advanced lesions in the aorta, coronary arteries, and stomach. 2. Concomitant renal hypertension was not alleviated by the presence of alpha-tocopherol in excess. In normotensive rats large dosages of this compound may induce moderate hypertension.

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## Inhibitory Effect of 5,6-Dimethylbenzimidazole and of 1,2-Dimethyl, 4,5-Diaminobenzene on Growth of *Mycobacterium tuberculosis.** (20971)

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(Introduced by Robert G. Bloch.)

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The incidence of pernicious anemia has been reported to be less in patients with active pulmonary tuberculosis than in the general population(1). It occurred to us that this observation might reflect vit. $B_{12}$ synthesis by the infecting tubercle bacilli, with consequent masking of any clinical manifestations of pernicious anemia.† Advances in the application of biologic antagonism to anti-bacterial therapy led us to investigate the influence of vit. $B_{12}$ fragments on the growth of *Mycobacterium tuberculosis*. The primary objective was to determine whether blockade of vit. $B_{12}$ synthesis or utilization in the tubercle bacillus might deprive the organism of an essential metabolite and thus halt its growth.

Two compounds, 5.6-dimethylbenzimidazole and 1,2-dimethyl 4,5-diaminobenzene‡ have been described by some as precursors of vit. $B_{12}$(3), and by others as vit. $B_{12}$ antagonists(4). The present report is concerned with preliminary observations of their effect on the growth of a virulent strain of *Mycobacterium tuberculosis* in Dubos Medium.

*Method.* Twenty screw-capped tubes, containing 6.1 ml of Dubos Medium and inoculated with roughly equal numbers of tubercle bacilli (0.1 ml of inoculum), served as controls. A similar quantity of such tubes, containing in addition, one of the various concentrations of 5, 6 D or of 1, 2 D, served as the experiment. All concentrations were simultaneously tested. In detail, actively growing, 6-day-old cultures of the H37Rv strain of *Mycobacterium tuberculosis*, which had been subcultured at least 3 times in Dubos' Tween-Albumin Medium(5), were employed as inoculum. Proportionate amounts of the basal culture medium and the inoculum, as well as the glucose and the bovine albumin, were pooled, to insure uniformity of concentration of the constituents in the many culture tubes. Subsequently, 5.1 ml of the suspension were added to each of the previously sterilized tubes, which contained either 1 ml of basal medium plus the compound tested or 1.1 ml of the basal medium for the control tubes. The final concentrations of 1, 2 D and of 5, 6 D were achieved by adding appropriate 0.1 ml aliquots of their aqueous solutions to lots of 20 tubes of inoculated medium. Because the 1, 2 D solution was found to be somewhat unstable, particularly when heated, it was sterilized by passage through a bacterial filter prior to the addition of the inoculum. The

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† More recent evidence has supported our hypothesis that vit. $B_{12}$ may be synthesized by *Mycobacterium tuberculosis*(2).

‡ Hereafter referred to as 5, 6 D and 1, 2 D respectively.