view of the favorable effects on osteomalacia, two of our patients with renal osteodystrophy received by mouth A.T.10 in 3 cc daily doses for 5 four-day metabolic periods while on a high calcium and moderate phosphorus intake. In both cases there was an immediate and progressive decrease of fecal calcium. While calcium appeared in significant amounts in the urine in one case, it remained absent in the other. The net retention of calcium at the height of A.T.10 action during the last period of its administration or the following period amounted to 50 per cent. of the intake. This was followed by a corresponding phosphorus gain due to a diminution of phosphorus elimination both in the stool and in the urine. The serum calcium, low initially in both cases, was raised to normal; and the inorganic phosphorus, high to start with, was reduced to normal during the A.T.10 therapy. Thus in remedying the basic metabolic defect underlying the bone disease in renal osteodystrophy, dihydrotachysterol appears to be highly efficacious, similar to vitamin D in rickets and osteomalacia. However, the effect of A.T.10 lasts for 7 or 8 four-day periods after the therapy is discontinued, in contrast to the long-sustained aftereffect of vitamin D in rickets and osteomalacia. Therefore, to secure substantial remineralization of the skeleton in renal osteodystrophy it would be necessary to administer A.T.10 for a prolonged period of time.

Another mode of therapy which we believe to be of interest in renal osteodystrophy is the oral administration of iron salts. It is well known in elementary chemistry that iron combines with phosphate to form insoluble ferric phosphate. That similar reaction takes place in the intestine is indicated by the experimental work[4] showing that iron added to a non-rachitogenic diet of rats produces rickets. Thus iron in large doses is contraindicated in rickets and osteomalacia. However, in renal osteodystrophy with hyperphosphatemia and high concentration of phosphate in the intestine interfering with the assimilation of calcium, the phosphate-precipitating action of iron may be utilized to advantage. Accordingly, the two patients wtih renal osteodystrophy referred to above were given ferric ammonium citrate 6 gm daily for from 5 to 14 metabolic periods. The most consistent changes were a decline of the serum inorganic phosphorus and an ascending tendency of the serum calcium. The phosphorus balance showed a decline due to an increase of stool excretion of phosphorus. The fecal elimination of calcium was usually diminished, giving rise to favorable calcium balance. This increase of calcium retention is most probably the result of the calcium-sparing action of iron in combining with phosphorus in the intestine. Thus from the standpoint of combating phosphate retention and promoting calcium gain in renal osteodystrophy, iron therapy proves effective.

In view of the present unsatisfactory state of affairs in the therapy of renal osteodystrophy, dihydrotachysterol (A.T.10) and iron seem to be rational and useful items in the treatment of such condition. As far as we are aware, the use of A.T.10 or iron in osseous disorder due to renal insufficiency has not been recorded in the literature. This is a preliminary report, and the detailed data will be published elsewhere.[5]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CONCERNING THE NATURE OF TYPE C BOTULINUS TOXIN FRACTIONS

THE first portions of condensate obtained by use of the standard lyophil apparatus in the dehydration of type C botulinus toxin consist of a high concentration of the thermo-stable fraction of this toxin. Recognition of this fraction in botulinus toxin was announced by Bronfenbrenner and Schlesinger in SCIENCE in 1921, though they gave no method of obtaining it in pure form in quantities sufficient for our study purposes.

This fraction, which for convenience may be designated as A, consists of ammonia salts. It is thermostable, and may be obtained in high concentration in almost pure aqueous solution by the method named. No antigenic property has been demonstrated for this fraction and it, therefore, has no specific antibody. Neutralization by type C antitoxin does not occur. Fraction A is a neuro-toxin which acts without delay. Sub-lethal intraperitoneal doses in mice result in nervous irritability for about 30 seconds, followed by what appears to be a complete anesthesia for four to six hours and eventual complete recovery. Thirty intraperitoneal, 18 gram mouse, mld's, administered orally to a three-pound mallard duck, result in a typi-

cal case of botulism. The onset in the duck is rapid with paralysis of the third eyelid and complete paralysis within one hour. Complete recovery may occur as early as the 24th hour, though 48 hours is the more common period.

Fraction A is volatile and escapes from an open vessel on prolonged boiling. It is very stable at room temperature and resistant to bacterial action as opposed to the thermo-labile fraction. Non-sterile, corked samples have been held for a ten-month period at room temperature without loss of toxicity.

It is unlikely that the A fraction ever exists in the free state in nature. Removal of this fraction from the toxin mixture as evolved by bacterial growth results in a remaining fraction which is no longer toxic by oral administration, though it is still toxic by injection. Restoration of the A toxin fraction regularly results in a return to toxicity by the oral administration of the mixture.

Fraction A is destroyed by strong alkali. This fact, coupled with the above findings, may account for the occasional collection of field samples, in semi-arid regions, which are toxic by injection in test animals but which are comparatively *non-toxic* by oral administration in normal doses. Complete separation of the two toxin fractions in nature has not been demonstrated. Reduction of fraction B to powder dryness by the lyophil process results in only partial loss of toxicity by the inoculation route.

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A DARKENING TECHNIQUE FOR INDUCING VIRUS SYMPTOMS IN MATURE AS WELL AS IN GROWING LEAVES

A RECENT article[1] on rapid transmission techniques for stone-fruit viruses was concerned with such devices as pruning, defoliation and girdling of growing plants for the purpose of shortening the incubation period. Incidentally, these techniques also offer promise for producing intensification of disease symptoms and for concentrating viruses in local areas.

Still more recently it has been discovered that the simple device of excluding light from the leaves into which one wishes to move the virus functions in a similar manner to the above but has the additional merit of inducing symptoms also on shoots and leaves that are not growing. To date only the virus of the Yellow-Red or X disease of peach has been tested, but it seems probable that the effects of shading will be generally applicable to other plant viruses.

Darkening the upper half of young rapidly growing peach seedlings for 2 weeks induced disease symptoms, sometimes within 4 weeks from the time the shades were installed. Similarly, darkening one of the branches on older seedlings which had completed their first season's growth induced symptoms within 6 weeks after the shades were installed. Although in these particular experiments the symptoms were not evident before 4 to 6 weeks after darkening, the shades need not be left on more than about 2 weeks and possibly the time of shading can be still further shortened. The growing seedlings were about 20 inches tall, branchless and each received a diseased bud midway on the stem. The older non-growing seedlings had either 2 or 3 branches and each received a diseased bud (sometimes with difficulty because of the cambium condition) near the base of the branches. Thus it was possible to darken either budded or unbudded branches. The shades, consisting of lightproof paper envelopes, had proper provision for ventilation and were held in place by clips attached to a stake.

The movement of the virus is apparently associated with the major movement of carbohydrate as pointed out by Bennett[2] for curly top virus movement in sugar beet and tobacco. Shading a portion of a plant stops photosynthesis in that part and favors the transport of carbohydrate into the shaded part, and if the entering food passes through a part of the stem containing the virus the latter apparently is carried along with the food. By placing the diseased bud somewhere between the shaded region and the food source, which in this case was unshaded leaves carrying on photosynthesis, it has been demonstrated that the virus was carried into the shaded leaves which were receiving food. Therefore, the fact that temporary darkening will induce entrance of the virus and the development of disease symptoms in non-growing as well as in growing tissues affords another important transmission technique to expedite investigations on plant viruses. Since darkening does not involve severe treatment of the plants nor necessitate new plant growth for symptom expression it should prove of value in many situations where pruning, defoliation and girdling can not satisfactorily be used or where it is difficult to induce the formation of new shoots.

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[1] E. M. Hildebrand, SCIENCE, 95: 52, 1942.

[2] C. W. Bennett, *Jour. Agr. Res.*, 54: 479–502, 1937.

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