Bilateral nephrectomy was performed on one of the rats of 22 pairs at the time of parabiosis. The pairs then lived for 3 to 11 days before the nephrectomized rat died. To insure that the coelio-anastomosis was of importance in maintaining life, parabiosis of a nephrectomized rat with a normal mate was done in 6 pairs without communication of the body cavities. All of these pairs died in 3 days or less, as did the single nephrectomized rats (Fig. 1).

Nonprotein nitrogen studies of whole blood showed that coelio-anastomosis was usually effective in maintaining low values, although shortly before death values of 100 to 200 mg % were sometimes reached. The nonprotein nitrogen of the nephrectomized rat usually remained between 75 and 100 mg % (Fig. 2) while that of the normal partner rose to 50 to 75 mg %.

The ability of the partner to excrete phenol red (PSP) injected into the nephrectomized rat was determined. Although there was negligible excretion in the urine when the rats were in parabiosis without coelio-anastomosis, good values were obtained with peritoneal anastomosis. After 5 mg of PSP were injected intramuscularly 0.1 to 0.2 mg was excreted by the partner in the first 6 hours. The values tended to increase when repeated after 5 days, probably due to the development of an anastomosis of the circulations.

In 15 experiments the peritoneal cavity of a single nephrectomized rat was continuously irrigated with Tyrode's solution. Although the life of the nephrectomized rat was not lengthened over that of the nephrectomized control the nonprotein nitrogen remained at values between 75 and 100 mg %. Absorption of fluid took place and death apparently was due to increased pleural fluid, and pulmonary edema.

*Conclusion.* These experiments demonstrated the efficiency of the peritoneal membrane as a mode of excretion in renal insufficiency. The effectiveness of the natural dialyzor provided by coelio-anastomosis suggests the feasibility of developing an artificial dialyzing mechanism.

## 15505 P

## "Dietary Factor" in Necrotizing Arteritis in Dogs a Lipid Substance.*

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During the past several years arterial lesions affecting principally the large elastic arteries (aorta, pulmonary artery, endocardium of the left auricle, and coronary arteries) have been produced with regularity by controlling 2 factors, diet and renal insufficiency.[1] The arterial lesions have been described and illustrated in previous publications, and the evidence for a dietary factor has been reviewed in a recent publication.[1]

In this paper is presented a summary of the data obtained from systematically testing the ingredients of the "standard diet" which was being fed at the time these unanticipated lesions were first encountered.

*Method.* The methods have been detailed in a previous publication.[1] Briefly, these consist of feeding a specified diet for a specified period of time (usually 8 weeks or longer), then damaging the kidneys (usually with heavy metal injury), and examining the arterial system both grossly and histologically when the animals die or are sac-

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1 Holman, R. L., *Am. J. Path.*, 1941, **17**, 359; *Am. J. Path.*, 1943, **19**, 977.

TABLE I.
Influence of Diet on Incidence of Arterial Lesions Following Kidney Damage.

| Diet | Weeks* | No. with lesions | |
|---|---|---|---|
| | | No. in group | % positive |
| Standard (6% C.L.O.) | 8-22 | 26/30 | 86.7 |
| Kennel | ? | 5/111 | 4.5 |
| Kennel + C.L.O. | 8-15 | 22/25 | 88.0 |
| Standard (corn oil substituted for C.L.O.) | 8-13 | 0/6† | 0.0 |

* Weeks of dietary feeding before production of kidney damage.
† One dog in this group had extensive necrotizing arteriolitis.

rificed (usually days or weeks after the renal injury).

"Standard diet," the diet that was being fed at the time these unanticipated lesions were first encountered, consisted of calves' liver (raw wet weight), 32 parts; cane sugar, 25 parts; corn starch, 25 parts; butter, 12 parts; and commercial cod liver oil (USP XI—vitamin A, 850 I.U. per g, and vitamin D, 85 I.U. per g), 6 parts. Enough tomato juice was added to make a paste of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per kg per day. Five grams of kaolin and 1 g of salt mixture were added to each day's diet. Essentially, this is a low protein diet with only 7% of its caloric value derived from protein, 43% from fat, and 50% from carbohydrate. With the "alterations" in this diet, *i.e.*, doubling, halving, or omitting different ingredients of the diet, care was taken to keep it isocaloric.

"Kennel diet," unless otherwise specified, consisted of selected table scraps from the University dining halls.

Both of these diets were kept in a refrigerated room (38-40°F) and were fed in a reasonably fresh condition. In other words, there was no reason to suspect rancidity or other forms of spoilage.

Renal injury was usually produced by minimum lethal dosage of heavy metal which, in the case of uranium nitrate, consisted of 5.0 mg of uranyl nitrate per kg of body weight injected subcutaneously in the hypochondrium in 0.5% aqueous solution, and in the case of mercuric chloride, consisted of 3.0 mg per kg body weight injected intravenously in one of the external jugular veins in 0.1% aqueous solution.

*Results.* In this preliminary report the detailed data obtained from doubling and halving the various ingredients of the standard diet are omitted, and all of the pertinent data to date are combined in one simplified table (Table I). Suffice it to say that all the negative as well as all the positive data that have been obtained point to something of lipid nature that (in dogs at least) has to be fed for a period of 8 weeks or longer before experimentally-induced kidney damage is regularly followed by arterial lesions.

Analysis of the data in Table I yields 3 types of evidence: (1) definite evidence for a dietary factor; (2) evidence that the dietary factor is contained in commercial cod liver oil; and (3) evidence that the dietary factor is not unique to cod liver oil—the finding of typical arterial lesions in 5 control dogs, *i.e.* dogs fed only kennel diet for an indefinite period before their kidneys were damaged, all occurred during an 18-month period when kennel diet consisted of beef bones with much adherent fat;[2] and the dog fed corn oil instead of cod liver oil that had extensive necrotizing arteriolitis is additional evidence that the dietary factor is not unique to cod liver oil. All the data implicate a substance (or substances) of lipid nature. The dietary factor is heat stable, is not readily oxidized, is not vitamin A, and is not vitamin D. Studies designed to further identify the dietary factor are in progress.

*Comment.* There is one important difference between these studies and previous

[2] Holman, R. L., *J. Exp. Med.*, 1945, **81**, 399.

ones[3] on arterial lesions related to cod liver oil—*renal insufficiency*. The manner in which renal insufficiency is produced (heavy metal injury, bilateral nephrectomy,[4] *Leptospira canicola*[5] is relatively unimportant, but some degree of renal damage is *essential*. The "standard diet" or kennel diet plus cod liver oil can be fed to dogs indefinitely (at least as long as a year) and no arterial lesions are ever observed until renal function is disturbed. This emphasizes the role of the kidney in the internal metabolism of the "dietary factor," which presumably is lipid in nature.

As time goes by, we gain confidence that arterial lesions can be produced with regularity in dogs by controlling 2 factors, (1) diet and (2) kidney damage; that both factors are necessary, and that only these 2 factors are involved.

[3] Agduhr, E., and Senstrom, N., *The Appearance of the Electrocardiogram in Heart Lesions Produced by Cod Liver Oil Treatment*, Uppsala, Almguist and Wiksells, 1930; Cowdry, E. V., *Arteriosclerosis*, New York, The Macmillan Co., 1933.

[4] Holman, R. L., *Am. J. Path.*, 1943, **19**, 159.

[5] Holman, R. L., unpublished data.

## 15506

## Pigment Studies on the Incisor Teeth of Vitamin E Deficient Rats of the Long-Evans Strain.*

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Several investigators[1-5] have reported that vitamin E deficient rats, over a period from 45 to 222 days, lose their natural brownish yellow pigment of the maxillary incisor teeth. During the past 9 years we have had occasion to examine several hundred vitamin E deficient rats of the Long-Evans strain. Although we did not check specifically for abnormal white incisors, we feel reasonably sure that such an obvious change in the pigmentation of the teeth would not have passed entirely unnoticed. To check accurately this depigmentation phenomenon in our strain of rats, the following experiment was conducted.

*Procedure.* Four groups (Groups 3, 5, 9, 11) of rats were maintained on our vitamin E deficient Diet No. 5† for either 310 or 460 days. Other groups (Groups 1, 7) were given Mason's vitamin E deficient Diet No. 69‡ for 130 days. Each of these groups was compared with normal control groups fed a commercial dog biscuit diet plus bi-

* Aided by a grant from the Milbank Memorial Fund of New York. The following materials were generously contributed: brewers' yeast by The Vitamin Food Company of Newark, N.J., and the cod liver oil by E. R. Squibb and Sons of New York City.

[1] Dam, H., and Granados, H., *Science*, 1945, **102**, 327.

[2] Davis, A. W., and Moore, T., *Nature*, 1941, **147**, 794.

[3] Granados, H., and Dam, H., *Science*, 1945, **101**, 250.

[4] Granados, H., and Dam, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 295.

[5] Moore, T., *Biochem. J.*, 1943, **37**, 112.

† Diet No. 5:

| | |
|---|---|
| Casein (commercial) | 24.0 |
| Cornstarch (uncooked) | 35.0 |
| Salts No. 2 | 5.0 |
| Lard | 20.0 |
| Cod Liver Oil | 2.0 |
| Brewers' Yeast | 10.0 |
| Cellulose Flour | 4.0 |

All diet ingredients, except the cod liver oil, were mixed and then allowed to stand at room temperature for 2 weeks. The cod liver oil was added just before feeding.