0.5 mc of P^{32}, were performed. Bone was partially decalcified with formic and nitric acids. Those sections decalcified with formic acid gave satisfactory autographs and demonstrated the retention of lead when dipped into a dilute solution of yellow ammonium sulfide. Those decalcified with nitric acid retained neither P^{32} nor lead.

Preliminary observations on adult giant rabbit and dog bones, softened overnight in formic acid, indicate that, since the metabolic turnover of phosphorus is slower than in growing animals, much larger tracer doses and longer intervals between injection and sacrifice of the animals are required. Further investigation is required to determine whether the method is applicable to adult rabbits and dogs.

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Lipemic Nephrosis in Rats[1]

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The experimental production of chronic renal disease by means of heteronephrotoxins was first reported by Lindemann (*3*). Masugi produced the disease in rabbits and described it as chronic glomerulonephritis (*4*). Smadel, Swift, and Farr (*7*) studied nephritis in rats induced by intravenous injection of antikidney serum obtained from rabbits and described it as a diffuse chronic and progressive glomerulonephritis. Smadel and Swift (*5, 6*), using rats of the Whelan, Evans, and Wistar strains, observed decreasing susceptibility to nephrotoxin and increasing capacity to recover from the initial nephrotoxic injury, in that order. Progressive glomerular disease was more severe in the Whelan than in the Evans and Wistar rats, and a high protein diet aggravated the course in the former and not in the latter strains (*7*).

We used the technic described by Smadel, Swift, and Farr with the following differences: (a) We used a Waring blendor for the preparation of kidney extracts; (b) we kept extracts and sera frozen for indefinite periods; (c) after addition of 1,000 units of penicillin/cc, some of our extracts were kept under toluene for 24 hrs at 37° C in an incubator; (d) the amount of serum given was not based on body weight but was calculated according to kidney weight; (e) we used rats of the Long-Evans strain exclusively; and (f) some rabbits were injected intramuscularly with extracts incorporated into an emulsion containing paraffin oil, a lanolin-like substance, and dry heat-killed tubercle bacilli (*1*).

[1] Aided by grants from the Life Insurance Medical Research Fund, the John and Mary R. Markle Foundation, and the Louis D. Beaumont Trust.

Sixteen rabbits were repeatedly injected intraperitoneally and 3 intramuscularly with renal extracts obtained from rats. The intraperitoneal treatment has thus far been more efficacious in producing nephrotoxic sera than has the intramuscular injection of extracts incorporated in Freund's adjuvants.

Six rabbits were treated with renal cortex, 7 with renal medulla, and 6 with extracts obtained from undissected kidneys. The separation of cortex and medulla was approximate and was done by sharp dissection. All of the sera obtained from rabbits treated with cortex and whole kidney extracts produced chronic renal disease in rats. Among the 6 sera obtained from rabbits treated with renal medulla, only 1 was about equally nephrotoxic, 2 produced a mild, transient proteinuria, and 3 were inactive. The ability of medulla extracts to produce nephrotoxic sera was not enhanced by injecting rabbits with a mixture of 2 parts of a 20% medulla suspension with 1 part of a 20% rat spleen suspension.

Renal disease produced by intravenous injection of the various nephrotoxic sera was obtained in 103 rats. The production of disease depended on dosage and on the individual susceptibility of the rats. Massive proteinuria was observed 1–2 days after intravenous injection of a nephrotoxic serum. When boiled with acetic acid, their urine often coagulated. Gross hematuria was not observed, and microscopic erythrocyturia was rare. A few leucocytes and numerous casts were usually present. Within the first and second week marked ascites and edema developed in 33 animals and persisted usually for 1–3 weeks. The natural course of the disease was observed in 35 rats. Spontaneous cures were observed in 13, a succession of remissions and relapses was seen in 12, and 10 rats showed a continuous, uninterrupted proteinuria for as long as 11 months. Blood pressure readings obtained in 42 unheated, nonanesthetized animals (*2*) varied between 90 and 125 mm Hg.[2] Forty animals remained normotensive during the course of their illness. In 2 rats hypertensive episodes with systolic values ranging between 130 and 145 mm Hg were observed $3\frac{1}{2}$ and $7\frac{1}{2}$ months, respectively, after onset of their disease.

Severe hypoproteinemia (lowest value, 1.6 gm/100 cc) and marked hyperlipemia (highest values, 1.96 gm/100 cc cholesterol and 19.5 gm/100 total lipids) were regularly observed in severely sick animals. In late stages of the disease a moderate degree of azotemia was observed only once. However, high nonprotein nitrogen values (100–200 mg/100 cc) were frequently obtained when rats were injected with lethal doses of markedly nephrotoxic sera. The blood pressure in all these animals was normal, and the highest concentration of creatinine observed in them was 2.6 mg%. High (40%) or low (5%) protein diets, otherwise isocaloric, were without influence on course or severity of the disease.

Histological examinations of kidneys and other tissues were obtained in 83 rats. The conspicuous renal change

[2] An apparatus was obtained from the Lederle Laboratories Division, American Cyanamid Company, through the courtesy of the late Y. SubbaRow.

was the presence of much protein in the nephrons. It was most abundant and deeply stained in the distal convoluted and collecting tubules but was also present in the proximal convoluted tubules and subcapsular spaces of glomeruli. The cells of the proximal convoluted tubules were usually swollen and granular and often showed hyaline droplet degeneration. The cells of the distal convoluted tubules showed similar but less striking changes. Special stains demonstrated fatty degeneration, notably of the convoluted tubules, and lipemia was noted in many cases. Slight degrees of chronic and subacute interstitial inflammation were present in 5 cases. There were no glomerular lesions except in 3 cases. These rats were killed, or died, 4, 5, and 8 days after onset. The capillaries were dilated with blood and with a hyaline coagulum. The tufts were comparatively acellular. Basement membranes were irregularly thickened in places but normal and even attenuated in others. In one instance a few neutrophilic polymorphonuclear leucocytes were present in the tufts and in the proximal convoluted tubules.

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Effect of Galactose on the Utilization of Fat[1]

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Schantz, Elvehjem, and Hart (*10*) reported that fat plays an important part in the utilization of galactose. They offered as evidence the observations that rats kept on a diet consisting only of skim milk excreted large amounts of galactose, and that the addition of butter fat (roughly in an amount equal to that of the lactose in the skim milk) reduced the excretion of galactose. Geyer, Boutwell, Elvehjem, and Hart (*7*) offered further similar evidence to prove this point.

With an entirely different technique, evidence was found in this laboratory that a reverse relationship exists between fat and galactose, namely, that galactose plays an important part in the utilization of fat.

For these experiments the so-called "single-food-choice technique" was used. Previous papers give full details (*8, 9*). It will suffice here to state that in the simplest form of this method rats of a standard weight and under standard conditions are placed on a diet that consists of only one foodstuff and water. The length of time the rats survive is taken as a measure of the nutritive value of the foodstuff. For example, it was found that without any food rats survived, on the average, only 4 days, whereas on galactose they survived 6 days and on glucose, 37 days. The significance of the results obtained with this technique depends on the observation that under these conditions rats seem to eat just as much of a purified foodstuff as they are able to utilize. This method has also been used, in a slightly more complicated form, to study the effects produced by various supporting substances. It was used, for example, to study the effect of thiamine on the utilization of glucose. To do this, the rats on a diet consisting exclusively of glucose were offered as a supplement 0.02% solution of thiamine hydrochloride. These rats survived, on the average, 76 days, or twice as long as on glucose alone, thus demonstrating beyond any doubt the remarkable effect that thiamine has on the utilization of glucose. In another form of this technique the interaction of foodstuffs on their mutual utilization can be studied by offering two foodstuffs at one time, as was done in the present experiments.

For these experiments domestic Norway rats were kept separately in cages that contained one or two nonspillable food cups and a graduated inverted bottle for water. The cages were made of wire cloth and were equipped with a large-meshed, wire-screen bottom to eliminate coprophagy.

Rats were started in the cages at ages of 38–47 days and kept on the stock diet until they were changed to the single-food diet. This occurred when they reached weights of between 120 and 150 gm.

In one series the rats had access only to galactose; in a second, only to oleo[2]; and in a third, to oleo and galactose (in separate containers). In a control series the rats had no food at all. On no food at all 15 rats survived from 3 to 6 days, with an average of 4.3 days. On galactose alone 13 rats survived from 4 to 8 days, with an average of 6.2 days; on oleo 10 rats survived from 19 to 38 days, with an average of 32.4 days; and on oleo and galactose 13 rats survived from 47 to 92 days, with an average of 69.3 days. This was over twice as long as on oleo alone, and over 11 times as long as on galactose alone.

Clearly, either the oleo must have had a great effect on the utilization of the galactose or the galactose must have had a great effect on the utilization of the oleo.

A comparison of the amounts of oleo and galactose eaten by the rats when they had simultaneous access to these two foodstuffs throws light on this relationship. The total caloric intake (average for the first 40 days) was 261.2 cal/kg/day, with the galactose contributing an average of 39.9 cal/kg, or only 15.3% of the total. For some of the rats the average daily galactose intake

[1] This study was carried out under a grant from the Sugar Research Foundation, New York City.

[2] Mrs. Filbert's, Baltimore, Maryland—vegetable oil, 80%; moisture, 15%; salt, 3.1%; skim milk, 1.5%; derivative of glycerine, 0.2%; sodium benzoate, 0.1%; vitamins from fish livers, 0.1%.