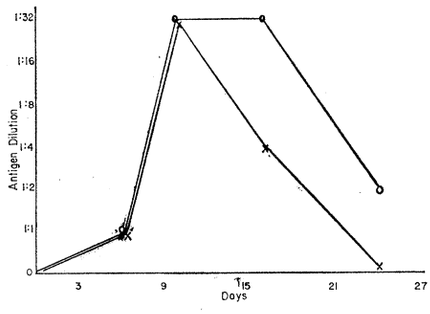

Fig. 1. Antibody response of irradiated animals. Ordinate, dilution of HGG from standard solution containing 1000 μg per milliliter; abscissa, days after challenging dose of HGG; (+) rabbit receiving 1 mc I^{131} TMV; (0) rabbit receiving ½ mc I^{131} TMV. Five animals, receiving 1.4, 1.6, 2, 2, and 4.4 mc, showed no antibody response and are not represented.

virus (TMV) very rapidly disappears (*7*) in a matter of minutes. Because of its ready availability and the rapid serum clearance this antigen was selected as the isotope carrier.

Tobacco mosaic virus (*8*) of the U-2 variety, maintained in cacodylate buffer, was dialyzed in 4-mg amounts against a solution of borate buffer at *p*H 8.0 and I^{131} was introduced by the iodine monochloride method (*9*).

Circulating TMV was detected by withdrawing samples of blood from the rabbits, performing a precipitin test on the separated serum, and measuring the radioactivity (due to I^{131}) in the washed precipitate. Within 1 hour, less than 10 percent of the TMV remained in the serum.

Five male Dutch rabbits were injected intravenously with 4 mg of TMV labeled with 5 μc of I^{131}. The animals were killed at intervals up to 8 days after injection. Samples of various organs were obtained and the I^{131} concentration was compared with that in the muscle in the same animal (Table 1). With respect to time, no definite pattern was seen. The relatively high concentrations in spleen, liver, lungs, and marrow are significant. A series of seven doses containing 0.5, 1.0, 1.4, 1.6, 2.0, 2.2, and 4.4 mc, respectively, was injected intravenously into the ear vein.

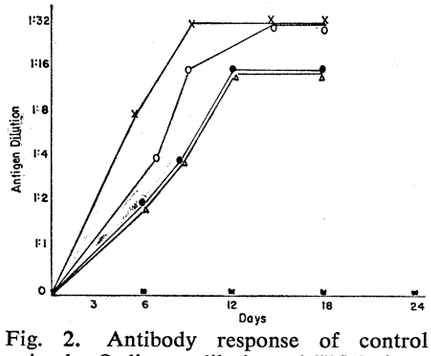

Fig. 2. Antibody response of control animals. Ordinate, dilution of HGG from standard solution containing 1000 μg per milliliter. Abscissa, days after the challenging dose of HGG. Each line represents antibody response of an individual animal. One animal showed no antibody response.

A total-body count was performed on one treated rabbit. Very little radioactivity remained after 10 days; it decreased during the first day to 40 percent and in the next 10 days to 10 percent.

Antibody titers to TMV were determined by the capillary-tube method in two of these animals on the 7th day after injection. No depression was seen or expected, since the major part of the irradiation should have been delivered several hours after administration of the I^{131}-TMV. According to Taliaferro (*10*) the radiation effect is most pronounced when the radiation is delivered one day prior to the antigen. At 6 to 7 days after I^{131}-TMV was given, the capacity to produce antibodies was tested by the administration of another antigen, human γ-globulin (HGG). Antibody titers to this test antigen were determined by the ring test. A cloudiness at the interface of the antigen layered on the antiserum within 2 hours was considered indicative of antibodies. After 7 days, all animals failed to produce detectable antibodies except those animals receiving 0.5 and 1.0 mc of I^{131}-TMV (Fig. 1).

All animals appeared to be in excellent health during the experiment. They ate well, gained weight, and showed no overt signs of illness.

The experiment was controlled by five Dutch rabbits from the same group to which TMV iodinated with nonradioactive iodine was given. They were given the test antigen in the same manner. All of these animals but one produced detectable antibodies to the test antigen (Fig. 2).

Although the number of animals tested was fairly small, there was a definite suppression of antibody formation detectable by the test described.

A very significant point is that no definite signs of radiation sickness or death occurred in these animals.

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Ethacrynic Acid: Diuretic Property Coupled to Reaction with Sulfhydryl Groups of Renal Cells

Abstract. *Ethacrynic acid, injected intravenously after surgical removal of one kidney of anesthetized dogs, lowered the concentration of protein-bound sulfhydryl groups in cells of the remaining kidney. Chloride excretion and urinary output of the kidney exposed to the drug increased. These findings indicate that the diuresis produced by ethacrynic acid may be related to its capacity for binding sulfhydryl groups of renal cellular proteins.*

Ethacrynic acid (Fig. 1) is a remarkable diuretic drug in dogs and humans. The drug and its biologically active congeners react with sulfhydryl groups in vitro (*1*). Moreover, effects of ethacrynic acid on urine flow and sodium chloride excretion closely resemble the effects of mercurial diuretics (*2*), compounds known to react with protein-bound sulfhydryl groups of renal cellular cytoplasm (*3*). These observations indicate that ethacrynic acid and mercurials may act in similar ways. As a first step in determining the way in

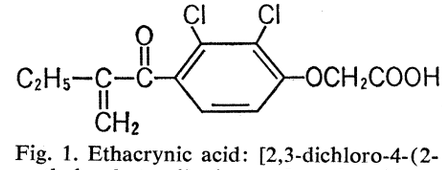

Fig. 1. Ethacrynic acid: [2,3-dichloro-4-(2-methylenebutyryl) phenoxy] acetic acid.

Table 1. Effects of ethacrynic acid (EA) on urine flow and chloride excretion. Data listed are mean values of output from the right kidney during 10-minute intervals before injection (control) and after injection (treated) of the drug.

Group	Urine flow (ml/min)	Chloride excretion (meq/min)
Control	6.6	0.43
Treated		
(0.5 to 3.0 mg EA/kg)	11.6	1.23
S.E. mean difference	1.4	0.15
p	<.01	<.01

which ethacrynic acid acts, we estimated the concentration of protein-bound sulfhydryl groups in renal cells and found it reduced in dogs that had been treated previously with ethacrynic acid.

Experiments were performed on seven mongrel dogs anesthetized with pentobarbital. Catheters were placed surgically into each ureter for separate collection of urine. The left kidney was removed and immediately afterward ethacrynic acid was administered intravenously. Twenty minutes later, when diuresis was maximal or increasing, the remaining kidney was removed. Thin slices of kidney tissue were fixed for 18 to 24 hours in 10 percent trichloracetic acid. Histological sections, 10 μ in thickness, were prepared and stained for protein-bound sulfhydryl groups by the method of Barrnett and Seligman (*4*).

Sections were then used for estimation of cytoplasmic protein-bound sulfhydryl groups of proximal and distal tubular cells, and medullary collecting-duct cells, by the method of Cafruny *et al.* (*5*).

In pilot experiments we discovered that the renal tubules of the diuresing right kidney were markedly dilated. For this reason, all animals included in this study were infused continuously with 10 percent mannitol at the rate of 1 ml/kg per minute. The ensuing osmotic diuresis caused maximum dilation of the tubules of both kidneys, thus insuring against spurious changes in the concentration of protein-bound sulfhydryl groups.

Table 2. Effects of ethacrynic acid (EA) on protein-bound sulfhydryl groups of renal cells. The results are expressed as the mean extinction value ± the standard error of the mean; kidneys from seven dogs were used.

Kidney region	Left kidney (control)	Right kidney (0.5 to 3.0 mg EA/kg)	p
Proximal convoluted tubules	0.641±.015	0.474±.007	<.001
Distal convoluted tubules	.591±.012	.494±.020	<.01
Medullary collecting ducts	.591±.026	.509±.015	<.02

Results are listed in Tables 1 and 2. The control group in Table 1 shows the average rates of urine and chloride excretion from the right kidney before administration of ethacrynic acid; the results obtained with the treated group shows that there was a significant increase in output of the right kidney following administration of ethacrynic acid.

Concentrations of protein-bound sulfhydryl groups, estimated spectrophotometrically as a colored complex with maximum absorption at 530 mμ, are given in Table 2. Values represent extinction [log(1/transmission)] determined through cylinders of cytoplasm (diameter, 2 μ; height, 10 μ). A total of 20 measurements in proximal and distal tubular cells were utilized for deriving a mean extinction value for each kidney; for the collecting ducts, 10 measurements were used. The standard deviation of these measurements did not exceed 12 percent of any mean value so derived. Extinction values listed in the table are averages of seven kidneys, 12 degrees of freedom being considered permissible in applying Fischer's "t" test for comparing two populations. The left kidney, removed before treatment with ethacrynic acid, served as a control.

A decrease in protein-bound sulfhydryl groups occurred in cells of the right kidney which had been exposed to a single injection of ethacrynic acid. Data are grouped even though the dose of ethacrynic acid ranged from 0.5 to 3.0 mg/kg, because the changes in renal sulfhydryl groups were of the same magnitude, as were changes in urine and chloride excretion (see Table 1), at each dose level. Cafruny *et al.* (*6*) have shown previously that concentrations of protein-bound sulfhydryl groups in the right and left kidneys of normal dogs do not differ.

These data support the premise that the mechanism of action of ethacrynic acid may be similar to that of mercurials. However, the drugs may differ with respect to site of action. Ethacrynic acid affects the protein-bound sulfhydryl groups of distal tubular cells; mercurials do not.

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Contraction-Band Formation in Barnacle Myofibrils

Abstract. *Contractions induced by adenosine triphosphate, in myofibrils isolated from the barnacle,* Balanus aquilia, *were observed with a phase microscope. The formation of contraction bands was well under way before the A-band came into contact with the Z-membrane. This is in disagreement with the hypothesis that contraction bands are formed when the A-band pushes against the Z-membrane.*

In a study of the changes which take place in myofibrils during contraction induced by adenosine triphosphate (ATP), we used the barnacle, *Balanus aquilia.* This species is similar to *B. nubilius*, which was described by Hoyle and Smyth (*1*) and shown by them to have especially large single fibers. In *B. aquilia*, both the single fibers and the individual myofibrils are large. The average length of the sarcomeres in the myofibrils is 9.2 μ, which is approximately four times the length of those in rabbit psoas muscle. The large sarcomeres in *B. aquilia* make certain features of the contraction of these myofibrils resolvable with the phase microscope.

A recent study by Hoyle and McAlear (*2*) resulted in the contention that the A-filaments of the barnacle