Thus the apparent therapeutic advantage of the Aureomycin antimony complex or the tetracycline antimony complex over the Terramycin antimony complex is questionable. The 3 antibiotic antimony complexes were active following subcutaneous or intraperitoneal injection, but were toxic at doses of 50 mg/kg and higher.

Discussion. It appears probable that the schistosomacidal activity and toxicity of the 3 antibiotic complexes result almost entirely from their antimony content, and that variations in the efficacy of these compounds compared with the other antimony-containing preparations mentioned arise from differences in solubility, absorption, and dissociation. Such variations in activity further emphasize the need for additional investigation of organic antimonial compounds which are schistosomacidal upon oral administration.

Summary. The antimony trichloride complexes of Terramycin, Aureomycin, and tetracycline were active orally against *S. mansoni* infections in white Swiss mice. These compounds were no more active than tartar emetic or antimony trichloride.

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Effect of Liver Damage and Nephrectomy on Convulsant Potency of Pentylenetetrazol (Metrazol)* (21959)

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There is lack of agreement on the fate and excretion of pentylenetetrazol (Metrazol) in laboratory animals and man. On the basis of data obtained by *chemical procedures,* Tatum and Kozelka(1) concluded that Metrazol is detoxified by the liver in dogs and man, whereas Esser and Kuehn(2) reported that the major portion of the drug is excreted by the kidney in man. Recent studies in our laboratory(3) with radioactive Metrazol indicate that most of a given dose of the drug in rats is excreted by the kidney in an altered form. On the basis of data obtained by *biological procedures,* Dille and Seeberg(4) concluded that Metrazol is degraded by the liver in cats, whereas Voss(5) reported that the drug is largely excreted by the kidney in rats. In view of these conflicting reports, it was thought important to make a systematic study of the effect of liver damage and of nephrectomy on the convulsant activity of this agent. It was anticipated that such a study would contribute information on the relative role of the liver and the kidney in the detoxification and excretion of Metrazol. The results obtained provide the basis for this report.

Methods. Adult male albino mice obtained from the Carworth Farms (CF #1) and adult male albino rats obtained from the Holtzman-Rolfsmeyer Farms were used as experimental

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animals. They were maintained on Purina Laboratory Chow and allowed free access to food and water except during the actual experimental procedure. The convulsant potency of *intravenously* administered Metrazol† was determined in control, liver-damaged, and nephrectomized mice; the convulsant potency of *subcutaneously* administered Metrazol was determined in control, liver-damaged, nephrectomized, and sham-operated mice and rats. In the determination of the convulsant potency, groups of at least 8 animals were given various doses of the drug until at least 3 points were established between the dose required to convulse 16% of animals and that to convulse 84% of animals. The results obtained were then plotted on logarithmic probability paper and a regression line visually fitted to the plotted points. From this plot of the data, the dose of Metrazol which was convulsant to 50% of animals (CD_{50}) was determined and the 95% confidence limits were calculated by the method of Litchfield and Wilcoxon(6). Intravenously, the drug (0.5% aqueous solution) was injected rapidly into the dorsal tail vein of the mouse; the endpoint employed was either a minimal seizure (any overt clonic activity which persisted for at least 5 seconds), or a full maximal seizure as indicated by the presence of the hindleg tonic extensor component(7). Subcutaneously, the drug (0.5% aqueous solution in mice, 5.0% in rats) was injected into a loose fold of skin over the neck; the endpoint employed was the occurrence of a minimal seizure, as described above, within one hour after the injection. Liver damage in mice was produced by a modification of the method of Stowell and Lee (8), *i.e.*, by the oral administration of 4 ml/kg of a 40% (w/v) solution of carbon tetrachloride (CCl_4) in olive oil. Maximum hepatic damage occurs about 100 hours after such administration and therefore Metrazol was tested at this time. Liver damage in rats was induced by a single subcutaneous injection of 2 ml/kg of a 50% solution (w/v) of CCl_4 in peanut oil. Histological studies of livers taken from rats treated in this manner have shown that maximum central and midzonal necrosis occurred approximately 48 hours after the injection of CCl_4; accordingly, Metrazol was tested at this time. Bilateral nephrectomy was performed in both species by the retroperitoneal approach while the animals were under light ether anesthesia. In order to keep the effect of accumulated metabolic products relatively constant, Metrazol was tested 6 and 12 hours after nephrectomy in mice and rats, respectively. Sham operation was performed by the same technic as for nephrectomy, except that the kidneys were not disturbed.

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TABLE I. Effects of Liver Damage and of Nephrectomy in Mice on Convulsant Potency of Intravenously Administered Pentylenetetrazol (Metrazol).

Exp. procedure	Convulsant potency (CD_{50}), mg/kg (intrav.) Max seizures	Min seizures
Control	29.0 (27.1-31.0)	21.0 (19.3-22.9)
Liver damage	29.0 (26.8-31.4)	19.5 (17.4-21.8)
Nephrectomy	28.5 (27.0-30.1)	24.5 (22.7-26.5)

Values in parentheses represent 95% confidence limits.

Results. The effects of liver damage and of nephrectomy in mice on the convulsant potency of intravenously administered pentylenetetrazol (Metrazol) are shown in Table I. The CD_{50}s for *intravenous* Metrazol for *maximal* seizures determined in liver-damaged mice and nephrectomized mice were not significantly different from the CD_{50} determined in control animals. On the other hand, the CD_{50} for *intravenous* Metrazol for *minimal* seizures, a more sensitive test, tended to be lower in liver-damaged mice and higher in nephrectomized mice. In view of these observations, it was decided to determine the CD_{50} of Metrazol administered by a route which would provide a longer time interval before the onset of convulsions than that provided by the intravenous route. It was anticipated that the longer sojourn of Metrazol in the body would induce more marked differences in the CD_{50}s determined in liver-damaged and nephrectomized animals, and thus delineate the relative

TABLE II. Effect of Liver Damage and of Nephrectomy in Mice and Rats on Convulsant Potency of Subcutaneously Administered Pentylenetetrazol (Metrazol).

Exp. procedure	Convulsive potency (CD_{50}), mg/kg s.c. route—min seizures	
	Mice	Rats
Control	59.0 (55.2-63.1)	47.0 (43.9-50.3)
Liver damage	46.5 (43.3-50.0)	39.5 (36.4-42.9)
Nephrectomy	66.0 (59.5-73.3)	45.5 (41.9-49.6)
Sham operation	65.0 (59.6-70.9)	42.0 (38.7-45.6)

Values in parentheses represent 95% confidence limits.

importance of these organs in the fate and excretion of the drug. Accordingly, Metrazol was injected *subcutaneously* in mice and rats and the CD_{50} for *minimal* seizures determined. The results obtained are summarized in Table II. The CD_{50}s for subcutaneous Metrazol are significantly lower in liver-damaged mice (21%) and rats (18%) than the control CD_{50}s. In sharp contrast, the CD_{50}s for subcutaneous Metrazol in nephrectomized mice and rats were not significantly different from the CD_{50}s in control or sham-operated animals. These data suggest that the liver plays the major role in the metabolic degradation of Metrazol in both species.

Discussion. The fact that CCl_4-induced liver damage and nephrectomy in mice had no significant effect on the intravenous CD_{50} of Metrazol is easily explained. The rapid intravenous injection of Metrazol elicits seizures so quickly that the organs concerned with the detoxification and excretion of this drug have only seconds in which to exert an effect. Hence, any influence which the liver or the kidney may exert is largely circumvented.

Liver damage lowered the CD_{50} for subcutaneously administered Metrazol by 21% in mice and 18% in rats. This suggests that the liver is important for the degradation of this drug. Additional evidence in support of this conclusion is the fact that fatalities after Metrazol were more frequent in liver-damaged than in normal mice. For example, in the dose range of 40 to 47 mg/kg, subcutaneous Metrazol was fatal to 37.5% of liver-damaged mice, whereas all normal mice survived the drug even in the higher range of 50 to 66 mg/kg. These observations are in agreement with the evidence that Metrazol is converted into an inactive product and that the site of this conversion is the liver(9).

Since there was no significant difference in the CD_{50}s of subcutaneously administered Metrazol determined in control, nephrectomized, and sham-operated mice and rats, it would appear that, within the time interval required to absorb the drug after subcutaneous injection, no appreciable amount of Metrazol is excreted by the kidney. This interpretation is in agreement with the observation of Esplin *et al.*(3) that less than 1% of intravenously administered radioactive (C^{14}) Metrazol is excreted by the rat kidney as an inactive product during the first 30 minutes, and that a total of 75% is excreted in an inactive form over a 48-hour period. Thus, the kidney appears to play a relatively minor role in the excretion of Metrazol or of an active conversion product.

Summary. The convulsant potency of intravenously administered Metrazol for maximal and minimal seizures was determined in control, liver-damaged, and nephrectomized mice. In addition, the convulsant potency of subcutaneously administered Metrazol for minimal seizures was determined in control, liver-damaged, nephrectomized, and sham-operated mice and rats. The results obtained were analyzed to determine the relative importance of the liver and the kidney in the fate and excretion of the drug. Neither liver damage nor nephrectomy had any significant effect on the potency of intravenously administered Metrazol for maximal or minimal seizures. Liver damage significantly increased the potency of subcutaneously administered Metrazol for minimal seizures; on the other hand, bilateral nephrectomy and sham operation had no significant effect on the potency of this drug. The data indicate that, in both mice and rats, the liver is the principal organ for the conversion of Metrazol into products devoid of convulsant activity, and that, within the time required for the complete absorption of subcutaneously injected Metra-

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Biological Method for Determination of Glucocorticoid Activity of Crystalline Compounds and Urine Extracts.* (21960)

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During the past decade, several biological methods for assay of compounds exhibiting glucocorticoid activity have been established. These technics have been applied to the assay of urine extracts to measure excretion levels of adrenal cortical hormone by the human. The methods of Reinecke and Kendall(1), Olson, Jacobs, Richert, Thayer, Kopp, and Wade(2), and Pabst, Sheppard, and Kuizenga (3), are based on capacity of glucocorticoids to deposit glycogen in livers of adrenalectomized rats after glycogen stores have been depleted by fasting. These methods use male rats weighing 140 to 180 g and injections of test material are made at regular intervals throughout the assay. The methods are reasonably specific for assay of glucocorticoids and reproducible with a fairly high degree of precision. The disadvantage of the methods lies in their comparative lack of sensitivity. Lack of sensitivity was apparently overcome by using the mouse as assay animal. The method of Venning, Kazmin, and Bell(4) measures deposition of glycogen in depleted livers of fasting adrenalectomized mice, while those of Eggleston, Johnston and Dobriner (5), and of Dorfman, Ross and Shipley(6) measure capacity of adrenal corticoids to prevent loss of hepatic glycogen during fasting. Our experience with the method of Venning, Kazmin and Bell demonstrated the assay to be unreliable with strains of mice available to us. Nissim(7), who conducted a critical study of various methods, believes that precision obtained by Venning *et al.* is dependent upon selection and careful control of a particularly suitable strain of mice bred in their own laboratories.

Existing methods were not suitable for our needs. The methods using young adult rats were not sensitive enough for assay of urine extracts, and uneconomical from the standpoint of animal cost and amount of test material necessary for assay. The mouse methods offered economy both in animals and test materials, but the strains of mice generally available did not lend precision to the assay. It therefore became desirable to develop a method based on satisfactory features of other methods. The method we devised is presented in this communication.

Materials and methods. The animals were 21-day-old male rats of the Holtzman strain weighing 50 to 55 g. These animals were

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