tidium caviae in some of the infected animals as well as in some of the controls did not appreciably alter the character of the normal mucosa. Whenever this ciliate was seen in the neighborhood of an amebic lesion, it did not appear to be in the advancing border of the lesion but was situated in the necrotic menstruum filling the crater of the ulcer. In rare instances it was seen in the subepithelial spaces. In the control animals and those reported as negative no ulcers of any kind were observed in the cecal mucosa; on the other hand, all the animals found to be positive by gross examination and by direct smear of the cecal feces, were found on histologic examination to have typical amebic lesions.

Summary. Guinea pigs were inoculated intracecally with graded single doses of from 100 to 5,000,000 amebae. None of the animals that were given less than 1,000 amebae developed infection. The 50% infective dose under the experimental conditions was 10,000 amebae. The mean weight of the guinea pigs that later became infected was significantly smaller than that of those which resisted infection. Thirty-one out of 50 guinea pigs that received single inocula of from 5,000 to 5,000,000 amebae died as a result of the infection. The LD_{50} under the experimental conditions was 63,000 amebae. Death occurred in all cases between 5 and 17 days after inoculation, on the average 9.3 days after inoculation, and the greatest number of guinea pigs died on the eighth day. In general, the size of the inoculum was found to have an inverse relationship to the average time between inoculation and death. Histopathologic studies confirmed necropsy examination. All animals found to be positive by gross examination and by direct smears of the cecal content were found to have typical amebic lesions, while in none of the controls and of those reported as negative were ulcers of any kind observed by histologic sections.

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Relationship Between Physiological Disposition and Adrenolytic Action of 2-(N-p'-tolyl-N-m'hydroxy-phenyl) - aminomethyl) - imidazoline hydrochloride (C-7337).* (17684)

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The relationship of physiological disposition to pharmacological action of adrenolytic agents has not been widely studied because of the lack of sensitive chemical tests for those agents which have been most extensively used up to the present time. The discovery of 2-(N-p'-tolyl-N-m'hydroxyphenyl-aminomethyl)-imidazoline hydrochloride (C-7337) has for the first time made such investigations possible since this compound may readily be determined in biological fluids and is a potent adrenolytic agent of low toxicity(1-5).

Chemical methods. A number of possible reactions involving the free phenolic group of C-7337 was investigated. The most satisfactory was found to be a modification of the 4-amino-antipyrine color reaction(6). The

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procedure as finally adopted for plasma, whole blood and urine was as follows: One ml of material to be analyzed was shaken mechanically with 0.5 ml of saturated sodium bicarbonate and 9.0 ml of chloroform for 10 minutes in a glass stoppered centrifuge tube. Sample was centrifuged for 3 minutes at 1000 RPM and the water layer removed by suction. A 7.0 ml aliquot of the chloroform layer was transferred to a test tube and chloroform removed by evaporation from a water bath. Four ml of distilled water were added to the residue and the sample heated for 5 minutes at 90-100°C to insure complete solution. On cooling, 1.0 ml of amino-antipyrine reagent (1.36 g of 4-amino-antipyrine to 1 liter of water) was added and mixed. One-half ml of alkaline ferricyanide solution (8.67 g of potassium ferricyanide and 1.8 ml of C.P. ammonium hydroxide made up to 1 liter with water) was added and the intensity of the resulting pink color was determined with a Beckman Spectrophotometer at 490 mμ. All samples were read between 5 and 10 minutes after final addition of the reagents which was the period of maximum development of color.

Approximately 85% of C-7337 added to water, plasma, and urine in concentrations of 1-100 γ/ml was recovered. As described, the method was not a suitable one for determining this drug in tissue since recoveries were poor and uncertain. This was especially true in the case of brain.

Preliminary studies indicated that approximately 10% was the maximum amount of C-7337 which could be recovered unchanged from the urine after intravenous administration. This observation led to a search for metabolic products. Attempts to extract material which coupled with amino-antipyrine from urine under acidic or basic conditions were unsuccessful. However, it was found that when urine was first hydrolyzed with 1.0 N hydrochloric acid and then brought back to the pH at which the free C-7337 had been extracted, approximately twice as much material was recovered. The procedure for the determination of "total" drug was as follows: 2.0 ml of urine were heated with 2 ml of 1.0 N HCl for 15 minutes in a boiling water bath. After cooling 4 ml of saturated sodium bicarbonate were added and 1.5 ml of the resulting mixture were shaken with 9 ml of $CHCl_3$ and treated as described for the determination of free C-7337.

General Plan of the Experiments. Dogs which had been fasted for 12 hours were secured to an operating table and prepared for intravenous injections and recording of blood pressure and pulse rate. After the response of each animal to control injections of U.S.P. epinephrine, C-7337 was administered and epinephrine responses retested, from time to time, until return to normal. Blood and urine samples were collected at appropriate intervals for determination of drug concentration. For the most part, animals were unanesthetized. However, in the case of some of the experiments in which it was necessary for dogs to be secured to the operating table for long periods of time, it was found necessary to administer morphine subcutaneously in a dosage of approximately 1.0 mg/kg. With the higher doses of drug, in which there were still adrenolytic action at the end of 20 hours, a number of experiments were carried out in which C-7337 was given the evening before test responses to epinephrine were begun.

Results. A series of 41 dogs was treated intravenously and orally with varying doses of the drug in order to determine the relationship between absorption, plasma concentration, excretion, and adrenolytic activity. The general kind of results obtained is illustrated by Fig. 1, which summarizes the results obtained following the administration of 15 mg/kg intravenously to a group of 5 dogs. Following this dose of C-7337 epinephrine reversal was produced for a period longer than 18 hours. In spite of this prolonged action, the drug disappeared rapidly from plasma and could not be detected after 1 hour (plasma level below 1 γ/ml). However, C-7337 continued to be excreted in the urine for as long as 24 hours, a total of 13.8% free C-7337 being recovered within a 24 hour period of observation. Thus it appears likely that C-7337 is extensively localized in tissues and it is this localization which determines the

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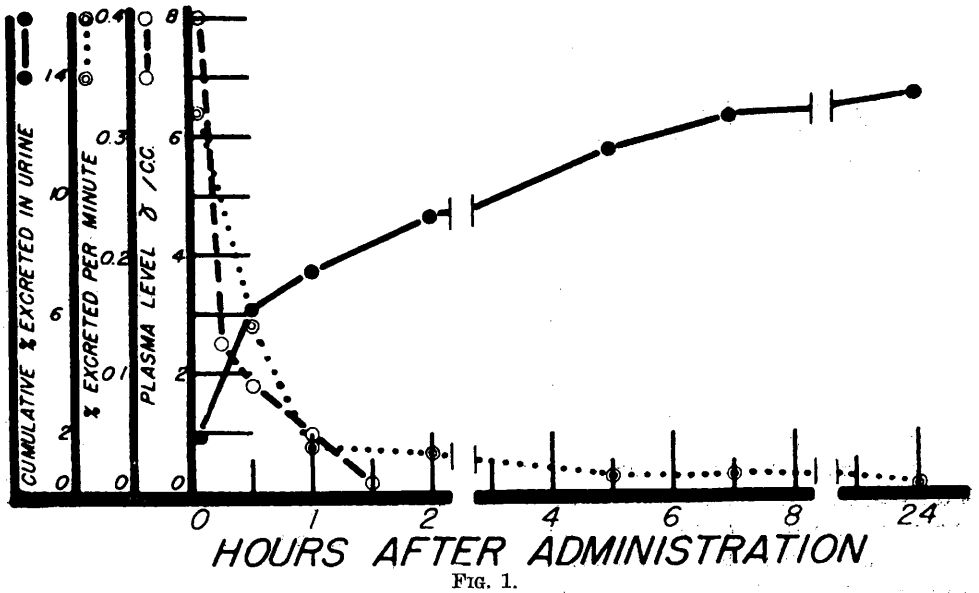

FIG. 1.

Plasma concentration and urinary excretion of C-7337 in terms of "free" drug after the intravenous injection of 15 mg/kg. Mean of 5 experiments.

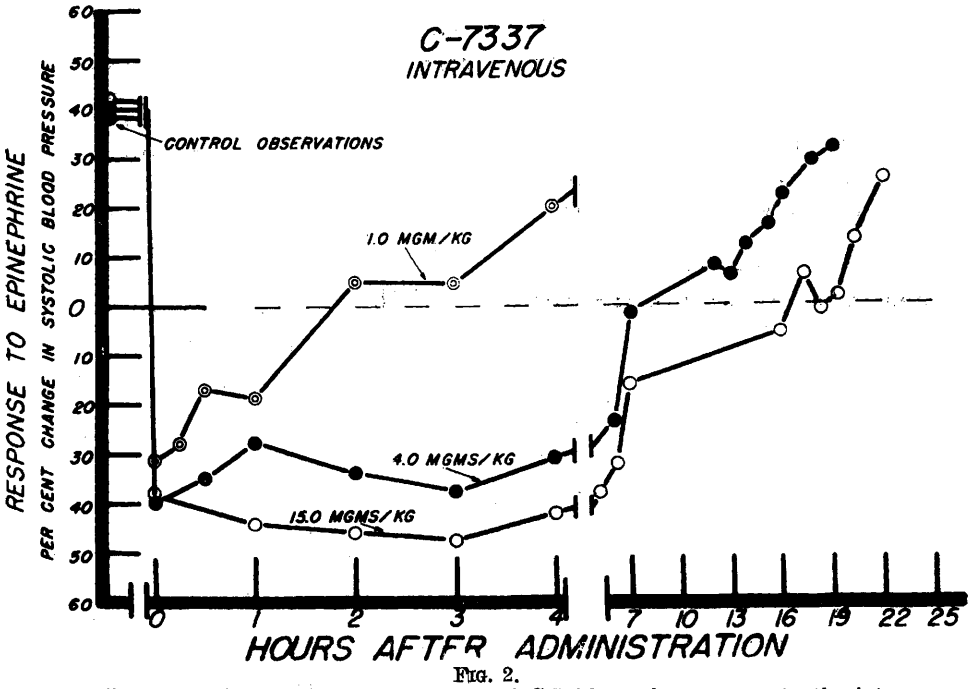

FIG. 2.

The effect of 3 different intravenous doses of C-7337 on the response to the intravenous injection of epinephrine.

degree and duration of adrenolytic activity.

Data which illustrate the relative effectiveness of oral and intravenous administration of C-7337 in blocking the pressor action of epinephrine are summarized by Fig. 2 and 3. It is apparent that increasing the dose of the adrenolytic agent increases the intensity of the response only up to a certain point

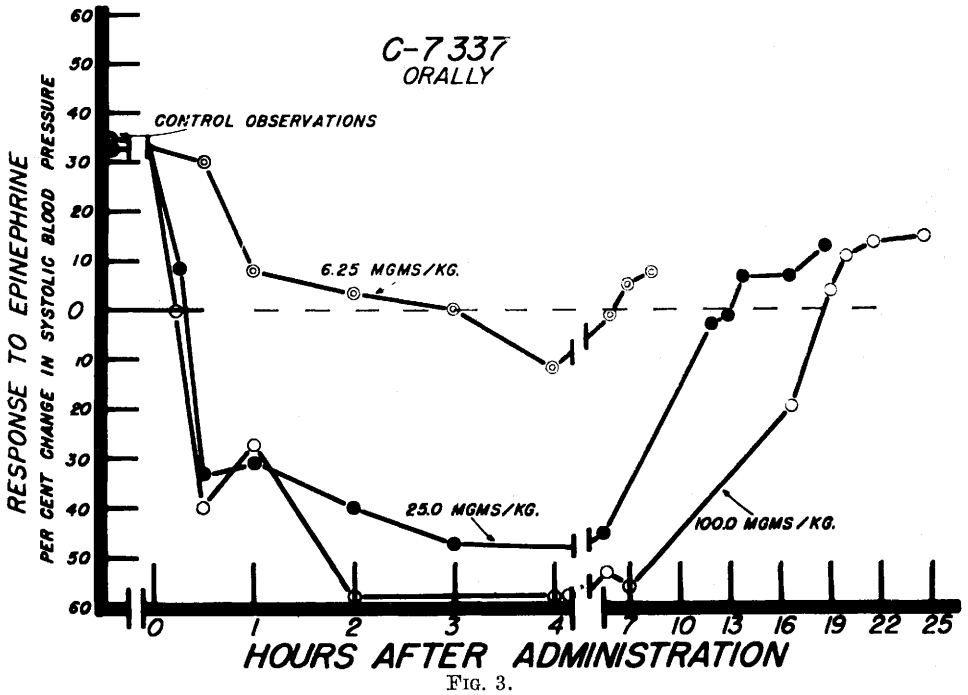

FIG. 3.

The effect of 3 different oral doses of C-7337 on the response to intravenous injection of epinephrine.

beyond which the effect of increasing dosage was manifest only by an increase in the duration of blockade. Comparing the effect of 1.0 mg/kg intravenously to 6.25 mg/kg orally it is also apparent that the injection of the drug causes a more intense blocking action than when the drug is introduced slowly into the circulation as by absorption from oral administration. In terms of maximum degree of action intravenous administration is many times more effective than oral administration. However, if the two routes of administration are compared as to duration of action, this difference is much less marked.

Table I summarizes relationship of adrenolytic activity to plasma levels and urinary excretion of drug. These data show that in terms of both maximum degree and duration of blockade, intravenous administration is approximately 6 times as effective as oral administration. The fact that peak plasma levels with oral administration were observed at 15 or 30 minutes would seem to indicate that absorption from the gastro-intestinal tract is rapid. With doses of 100 mg/kg orally the plasma concentration decreased more slowly from the peak level of 4 γ/ml than was seen after a single intravenous dose. The data in Table I also demonstrate that complete blockade and reversal of the pressor response of epinephrine may occur without plasma concentrations even reaching a level of 1 γ/ml. These findings may be explained on the basis of rapid localization within some tissue or body fluid. From the time-plasma concentration curves showing the disappearance of the drug from the blood following a single intravenous dose the apparent volume of distribution was estimated by extrapolating the curves back to zero time. This value also indicated extensive tissue localization.

Despite the fact that the per cent urinary excretion of total drug was generally greater after intravenous injection than after oral administration, a greater percentage of the total drug recovered was conjugated with oral administration (52-65%) than after intravenous injection (31-41%). This suggests that gradual absorption from the gastro-intestinal tract may favor conjugation.

Discussion. No definitive information has

TABLE I.
Summary of Data Showing Relationship of Plasma Concentration and Urinary Excretion to Adrenolytic Activity of C-7337.

Dose, mg/kg	No. of animals	% of dose recovered in urine		Blockade of epinephrine pressor effect		Mean peak plasma level, γ/ml	
		Free	Total	Onset min.	Duration in hr	Conc., γ/ml	Min. after administration
				Intravenously			
1.	7	8.5	12.4	<2	2	<1.0	—
4.	6	8.7	12.9	<2	10	8.7	0–5
15.	8	13.8	23.3	<2	18	8.3	0–5
				Orally			
6.25	6	2.6	5.4	120	2	<1.0	—
25.	7	4.9	13.9	15	12	1.2	15–30
100.	7	4.3	10.4	15	18	4.0	15–30

been obtained as to the metabolic fate of C-7337 or the nature of the conjugated material which can be detected in the urine. A small amount of impure hydrolyzed metabolic product has been collected and tested for its pharmacological action. The concentration of the material used for these biological tests was determined in terms of material which coupled with 4-amino-antipyrine. It was found that this metabolic product did possess adrenolytic action, although it was less than ¼th as active as an equal amount of pure C-7337 and was shorter in duration of effect. Since the material isolated was not pure the only conclusion which can be drawn from these experiments is that some of the metabolic products have lost their effectiveness as adrenolytic agents.

Summary. 1) A chemical method is described for the determination of C-7337 in plasma and urine which is sensitive to at least a concentration of 2 γ/ml.

2) In terms of duration of blockade of epinephrine pressor effect, C-7337 is approximately 1/5th as active by mouth as by intravenous injection.

3) Approximately 10% free C-7337 may be recovered in the urine following intravenous injection whereas only one half this amount may be recovered after oral administration.

4) C-7337 disappears rapidly from plasma but continues to be present in the urine for as long as 24 hours. These results indicate that the drug is extensively localized in some tissues of the body.

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Interference by Glucose in the Quantitative Determination of Uric Acid.* (17685)

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A notable increase in uric acid and glucose excretion was observed in a patient with juvenile rheumatoid arthritis (Still's disease) while receiving cortisone and also when given ACTH. The parallelism between observed values of uric acid and glucose was impressive and suggested the need to check the effect of glucose on the values obtained for uric acid by the method used. The method for the determination of uric acid was that of Buchanan, Block and Christman(1) in which uric

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