tion in the anterior pituitary and that the eosinophilic granulation is restored by treatment with thyroid hormone(12), suggests that the protein anabolic effect of thyroid hormone may be an indirect effect exerted by inducing growth hormone secretion of the anterior pituitary. The action of testosterone on the other hand must be a direct one, mediated neither through the anterior pituitary nor through the thyroid.

Conclusions. Testosterone propionate after an initial latent period of 3 days causes a decrease in the urinary nitrogen excretion associated with an increased weight gain in the thyroidectomized rat fed a constant intake. Escape during continued treatment, and a "rebound" after the cessation of treatment occurs in the thyroidectomized rat just as it does in the normal, castrated or adrenalectomized rat.

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Physiological Disposition of 2-Phenyl Quinoline-4-Carboxylic Acid (Cinchophen) in Man. Method for its Estimation in Biological Material. (21113)

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Cinchophen (2-phenyl quinoline-4-carboxylic acid) has been extensively used as an analgesic, antipyretic and uricosuric agent. However, its use has diminished in recent years because fatal hepatitis has occasionally occurred during therapy with this drug. In spite of the once widespread use of cinchophen, relatively little information exists concerning its physiological disposition and fate in man. The development of a specific and sensitive method for the estimation of cinchophen in biological material has made it possible to study the fate of the drug in man. Data concerning the absorption, excretion, rate of biotransformation and distribution will be presented in this paper.

Methods. Estimation of cinchophen in biological materials. Cinchophen is isolated from biological materials by extraction into benzene at pH 4.0. The drug is returned to pH 9.0 buffer, acidified and assayed spectrophotometrically at 345 mμ. *Procedure for plasma.* To one to 3 ml plasma in a 60-ml glass-stoppered bottle add an equal volume of 0.4 M citric acid and 30 ml benzene* containing 1.5% isoamyl alcohol.* Shake for 10 minutes and centrifuge the bottle. Transfer 20 ml of the benzene phase to another 60-ml glass-stoppered bottle containing 5 ml of borate buffer pH 9.0 and shake for 5 minutes. Transfer 4 ml of the aqueous phase to a

* Solvents (Reagent grade) are purified by successive washings with 1N NaOH, 1N HCl, and 3 washings with water.

cuvette; add 1.0 ml of 1N HCl and read the optical density at 345 mμ in a Beckman spectrophotometer. Reagents run through the above procedure are used for the zero setting. Standards are prepared by handling a known amount of cinchophen in the same manner as unknown samples. An optical density of 0.103 is obtained in a Beckman spectrophotometer when 10 μg of cinchophen are carried through the procedure. *Procedure for urine.* Proceed as with plasma, but wash the benzene extract 3 times with 1/5th volumes of pH 4.0 buffer.† *Procedure for tissues and feces.* Adjust 3 ml of homogenized tissue or feces‡ to pH 4.0 and proceed as described for plasma. Tissue and red cells contained normally occurring blank material which absorbed light at 345 mμ. Part of this material was removed by washing the benzene extract twice with 1/5th volume of pH 4.0 citrate buffer. The correction for the residual blank depended on the observation that its optical density at 345 and 410 mμ was essentially the same, whereas that of cinchophen was decreased by about 95%. The cinchophen concentration in the solution can be calculated by the following equation: $\frac{Ua - Ub}{Sa - Sb} \times C$. Where Ua and Ub are the optical densities of the unknown at 345 and 410 mμ, respectively, Sa and Sb are the optical densities of the standard at these wave lengths and C is the concentration of cinchophen in the standard. Cinchophen added to biological materials in amounts of from 5 to 100 μg was recovered with adequate precision (97 ± 4%). *Assay of specificity.* An examination of specificity for the estimation of cinchophen was made by the technic of comparative distribution ratios (1). Plasma and urine of a human subject who had received the drug were extracted in the same manner as described in the procedure. The partition coefficients of authentic cinchophen between benzene and a number of aqueous solutions at various pH values were compared with those of the apparent compound obtained from biological materials. The results showed that within experimental error the apparent and authentic compounds had the same solubility characteristics and were presumably the same compounds (Table I).

† The buffer wash serves to remove interfering material which absorbs light at 345 mμ.

‡ Organ tissues and feces are prepared for analysis by emulsification in acid as described previously(1).

TABLE I. Distribution of Cinchophen and Apparent Cinchophen between Benzene and Water at Various pH Values. The apparent cinchophen from plasma and urine of a subject who had received cinchophen orally was extracted into benzene at pH 4. The urine extract was washed 3 times with pH 4.0 buffer. Aliquots of the benzene extracts were shaken with one-half volume of several buffer solutions. Authentic cinchophen in benzene was treated in the same manner. The fraction of the compound extracted at various pH values is expressed as the ratio of the amount of compound in the organic phase to total compound.

pH	Authentic cinchophen	Apparent cinchophen	
		From plasma	From urine
5.0	.83	.83	.83
5.7	.65	.65	.64
6.2	.45	.47	.45
7.0	.16	.16	.17

Results. Absorption and excretion of cinchophen. Information concerning the absorption from the gastrointestinal tract and renal excretion of cinchophen was obtained from a balance study on 2 human subjects who received 500 mg of the drug orally. Urine and feces were collected over a 72-hour period and examined for cinchophen. Less than 1% of the drug was found in the feces, indicating that absorption from the gastrointestinal tract was essentially complete. The urinary excretion accounted for only 2% of the total dose, indicating that the drug was almost completely metabolized in the body.

Plasma concentration—time curves. Plasma concentrations of cinchophen were measured at various time intervals in 4 subjects who received single oral doses of 500 mg of the drug. Peak plasma levels were achieved in 1.5 to 3 hours after the drug was administered and then declined exponentially (Fig. 1). The biologic half-life of cinchophen, that is, the time required for the plasma drug level to decline by one-half, ranged from 4.0 to 4.5 hours, indicating a relatively uniform rate of biotransformation of the drug in these individuals. The rate of biotransformation of cinchophen is slower than other analgesic

drugs such as acetanilide(2), Acetophenetidin (3), and Aminopyrine(4), but faster than antipyrine(5).

Distribution of cinchophen. The degree to which cinchophen in plasma is bound to proteins was determined by dialysis against isotonic phosphate buffer of pH 7.4 at 37°C for 20 hours. Visking membranes were used for the dialysis bags. A definite relationship between plasma concentration of cinchophen and the degree of binding to the non-diffusible constituents of the plasma was found. At plasma concentrations of 10 mg per liter only 5% of the drug was present in the unbound form, whereas 27% of the compound was found free in the plasma at concentrations of 250 mg/liter. The distribution of cinchophen was examined in representative tissues of a dog which received 150 mg/kg of the drug orally. Two hours after drug administration, the animal was sacrificed by an intravenous injection of air and the tissues immediately sampled (Table II). With the exception of bile, the cinchophen concentration was highest in plasma. The amount of drug present in the plasma water was considerably less because of the high degree of drug binding to plasma protein. Although the concentration of the drug in cerebrospinal fluid was low, the concentration was of the same order of magnitude as that in the plasma water suggesting that there is little hindrance to the passage of drug across the blood-brain barrier. The concentration of cinchophen in most organ tissues was about half that of plasma. However, drug concentrations in organ tissues was higher than that of the plasma water, indicating definite localization of cinchophen in the tissues. The concentration of cinchophen in red cells was about one-fourth that found in plasma, suggesting that the drug is distributed to a considerable degree in the extracellular fluid.

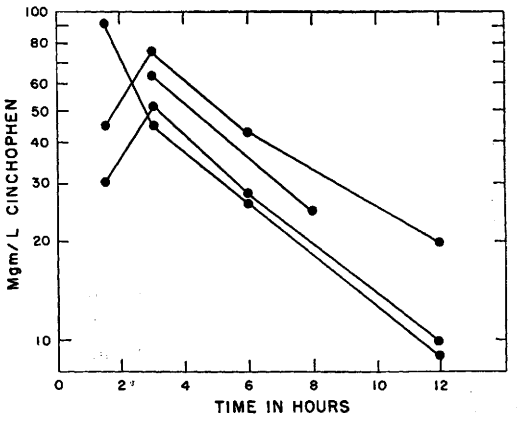

FIG. 1. Plasma levels of cinchophen in man after oral administration of the drug to 4 subjects.

TABLE II. Distribution of Cinchophen in Dog Tissues. The dog received 150 mg/kg of cinchophen orally. The tissues were examined 2 hours after administration of the drug.

Tissue	Conc. of cinchophen (mg/kg)
Plasma	340
Red cells	92
Cerebrospinal fluid	40
Bile	900
Liver	215
Lung	150
Muscle	79
Kidney	168
Heart	134
Brain	42
Spleen	90

Summary. A specific and sensitive method for the measurement of cinchophen in biological fluids and tissues is described. Cinchophen is essentially completely absorbed from the gastrointestinal tract. The drug is almost entirely metabolized in man, only about 2% being excreted in the urine. The rate of biotransformation of cinchophen is fairly uniform, among a number of subjects the average half-life being 4 hours. Cinchophen is localized in most organ tissues. The drug is largely distributed in extracellular fluid and at low concentrations is highly bound to plasma proteins.

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