by the administration of desiccated whole liver or its water-insoluble fraction but not by supplements of the various known nutrients (8-11). Results of the present experiment indicate that desiccated and defatted kidney and a fermentation product derived from cultures of *Streptomyces aureofaciens* (APF - 5) were as active as liver residue in counteracting the growth retardation of immature rats fed massive doses of desiccated thyroid. Full fat soybean flour also showed considerable activity in this regard. Stokstad *et al.* (13) have recently observed that aureomycin mash contains a factor apparently distinct from any of the known nutrients which is essential for the optimal growth of chicks. The activity of aureomycin mash under conditions of the present experiment suggests the possibility that the "antithyrotoxic factor of liver" and the chick growth factor of Stokstad *et al.* may be one and the same factor. Crystalline aureomycin HCl, however, which exerts growth-promoting activity in the chick(14). turkey and pig(15) is inactive as a source of growth-promoting factor for the hyperthyroid rat. The ineffectiveness of whey (Table I) appears to distinguish the active principle in the present experiment from the "whey factor" described by Hill(16). Some increase in body weight over that obtained on the basal ration was observed in thyroid-fed rats under conditions of the present experiment following the administration of desiccated and defatted heart, beef flank, thymus, placenta, duodenum or brain as well as powdered whole egg, fish meal, whey, alfalfa, yeast or streptomycin and penicillin mash. The gain in body weight of rats fed these supplements, however, was less than that of animals receiving an additional supply of the known B vitamins suggesting that the growth-promoting effect of these supplements may have been due, at least in part, to their content of known B vitamins.

Summary. Growth was markedly reduced in hyperthyroid rats fed a purified ration containing casein as the dietary protein and sucrose as the dietary carbohydrate. The retardation in growth was completely counteracted by the administration of a water-insoluble fraction of liver, desiccated and defatted kidney and a fermentation product derived from cultures of *Streptomyces aureofaciens*. Full fat soybean flour also showed considerable activity. Crystalline aureomycin HCl was inactive. Other materials of plant and animal origin showed little if any activity. The protective factor was apparently distinct from any of the known nutrients.

13. Stokstad, E. L. R., Jukes, T. H., Pierce, J. V., Page, A. C., Jr., and Franklin, A. L., *J. Biol. Chem.*, 1949, v180, 647.

14. Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 523.

15. Stokstad, E. L. R., Jukes, T. H., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B., *Arch. Biochem.*, 1950, in press.

16. Hill, F. W., *Poultry Science,* 1948, v27, 536.

Received May 12, 1950. P.S.E.B.M., 1950, 174.

Absorption, Distribution and Excretion of Phenindamine (Thephorin). (17919)

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The compound, phenindamine (2-methyl-9-phenyl 2,3,4,9 tetrahydro-1-pyridindene), is available under the trade name Thephorin for the treatment of various allergic conditions. Its antihistaminic and other pharmacologic properties have been described by

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Lehmann(1). However, despite its wide therapeutic usage, little is known in regard to its absorption, distribution and excretion. The results of such studies on rats and its urinary excretion in humans are presented in this report.

Procedure and results. Estimation of Phenindamine: The compound was determined quantitatively by the Brodie and Udenfriend methyl orange technic for basic amines (2) using technical benzene as a solvent. It was found necessary to modify the method for estimating phenindamine recovered from biologic samples because it became apparent in our preliminary studies that considerable material extracted by the benzene reacted as phenindamine in the analytical procedure. Previous experience with the estimation of other basic amines (Demerol(3), Methadone (4), and Pyribenzamine(5)) by the methyl orange technic suggested that interfering substances could be removed by simply washing the solvent extract with a buffer at an appropriate pH. With phenindamine, this was accomplished by washing the benzene extract of tissue 4 times with an acetate buffer at pH 5.5. Recovery of known amounts of phenindamine added to various tissue homogenates averaged 85 $\pm$ 10%. Tissue blanks averaged from 0.005 optical density for muscle to 0.025 for brain.‡

Procedure for Tissues. Weighed samples of the tissue to be studied were diluted 1 to 15 with M/50 sodium fluoride solution and thoroughly minced in a Potter homogenizer. One cc of N/1 NaOH and 10 cc of benzene were added to 5 cc of tissue homogenate in a glass stoppered centrifuge tube and shaken for 3-5 minutes. The mixture was centrifuged and the aqueous layer removed by aspiration. Five cc of M/2 acetate buffer pH 5.5 were added to the benzene layer. After shaking thoroughly, the buffer phase was removed by centrifugation and aspiration. The benzene layer was washed three additional times in the same manner. A 7 cc aliquot was transferred to a glass stoppered tube containing 0.5 cc of the methyl orange reagent. Isoamyl alcohol (0.25 cc) was added and the tube shaken for 1 minute. The tube was then centrifuged and the methyl orange layer completely removed by aspiration. The benzene layer was transferred to another tube and a 5 cc aliquot of this solution was added to a colorimeter tube containing 1 cc of 2% H_2SO_4 in absolute alcohol. The intensity of the color thus developed was read in a Coleman Junior Spectrophotometer at 540 mu and the amount of phenindamine present was estimated by reference to a standard curve.

Specificity of Method. Evaluation of the specificity of the method was carried out according to Brodie(2). An unknown compound may be identified with a known substance by comparing their distribution ratios between an organic solvent and a series of aqueous buffers of varying pH. Similar ratios would indicate that the two substances are alike since the probability is rather remote for 2 different compounds to have identical solubility properties. Accordingly, this principle was applied to the phenindamine recovered from various organs of several rats which were given the compound by the intraperitoneal route. After extraction of the minced tissue with benzene, an aliquot of the benzene layer was analyzed for methyl orange reactants. Aliquots of the same solution were equilibrated with a series of phosphate buffers and the amount of methyl orange reactant remaining in the benzene was determined. The ratios of the amount of methyl orange reactant remaining in benzene before and after equilibration with the buffers were compared with those obtained with a known sample of phenindamine.

From the results in Table I, it became obvious that the alkaline benzene extract of

1. Lehmann, G., *J. Pharmacol.*, 1948, v92, 249.
2. Brodie, B. B., and Udenfriend, S., *J. Biol. Chem.*, 1945, v158, 705.
3. Leong Way, E., Gimble, A. I., McKelway, W. P., Ross, H. and Ellsworth, H., *J. Pharmacol.*, 1949, v96, 477.
4. Leong Way, E., Sung, C. Y., and McKelway, W. P., *J. Pharmacol.*, 1949, v97, 222.
5. Leong Way, E., and Dailey, R. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 423.

‡ Benzene on standing may yield higher brain blanks and must be checked at frequent intervals for such changes.

TABLE I.
Distribution Ratios of Phenindamine Remaining in Benzene to Total Drug (Added or Recovered) After Equilibration with Various Buffers.

Sample description	No. of buffer washes	0.5 M phosphate, pH 8	7	6	5
Added to water	0	1.01	1.04	.99	.98
Recovered from liver	0	1.01	.99	.77	.59
" " kidneys	0	1.03	1.03	.87	.72
" " lungs	0	.89	.88	.79	.53
" " "	1	—	.99	.88	.59
		0.5 M acetate, pH 5.5	5.1	4.1	3.3
Added to water	2	.98	.97	.72	.36
Recovered from liver	3	.85	.80	.51	.29
" " "	4	—	.99	.71	.37
" " kidneys	3	.99	.91	.66	.32
" " lungs	3	.86	.84	.61	.37
" " "	4	—	.97	.68	.40
" " spleen	3	.98	.91	.73	.35

tissues contained material which behaved differently from phenindamine, since the distribution ratios of the methyl orange reactant recovered from animals differed considerably from those of a known sample of phenindamine. In all probability this was due to the presence of degradation products which reacted as the parent compound. It was subsequently found that the interfering substances could be removed by washing the benzene extract of tissues 3-4 times with M/2 acetate buffer, pH 5.5. After such treatment the solubility properties of the methyl orange reactant remaining in the benzene extract of tissues were found to resemble those of a known sample of phenindamine (Table I).

Absorption of phenindamine. The rate of oral absorption of phenindamine was studied by measuring its rate of disappearance from the gastrointestinal tract of rats fasted for 18-24 hours. A dose of 100 mg/kg was administered by stomach tube. The animals were sacrificed at various time intervals and their gastrointestinal tracts were removed, diluted to 500 cc and thoroughly minced in a Waring Blendor. The amount of drug still present in a sample of the homogenate was determined as previously described. It was assumed that destruction of the drug in the gut did not occur, since incubation studies of phenindamine (added or recovered) with the enteric tract plus its contents did not reveal any alteration of the original phenindamine concentration. The results as shown in Fig. 1 indicate that approximately 75% of a phenindamine dosage disappears from the gastrointestinal tract within 4 hours. Since approximately 10% of the dosage was still present in the gastrointestinal tract after 24 hours, excretion of the compound by the gut must be considered. In contrast, our previous results with another antihistaminic, Pyribenzamine, revealed that practically all of the compound had disappeared from the enteric tract 4 hours after oral administration (5).

Tissue distribution of phenindamine. The distribution of phenindamine was determined on rats. Following the administration of

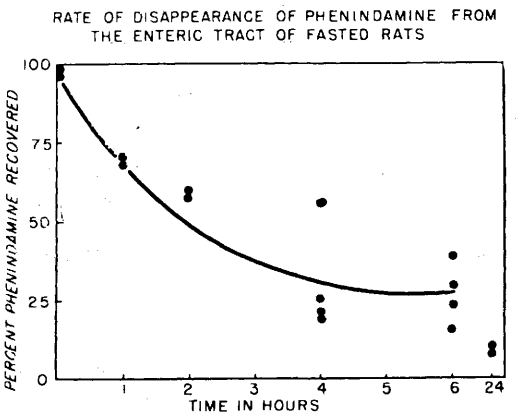

FIG. 1.

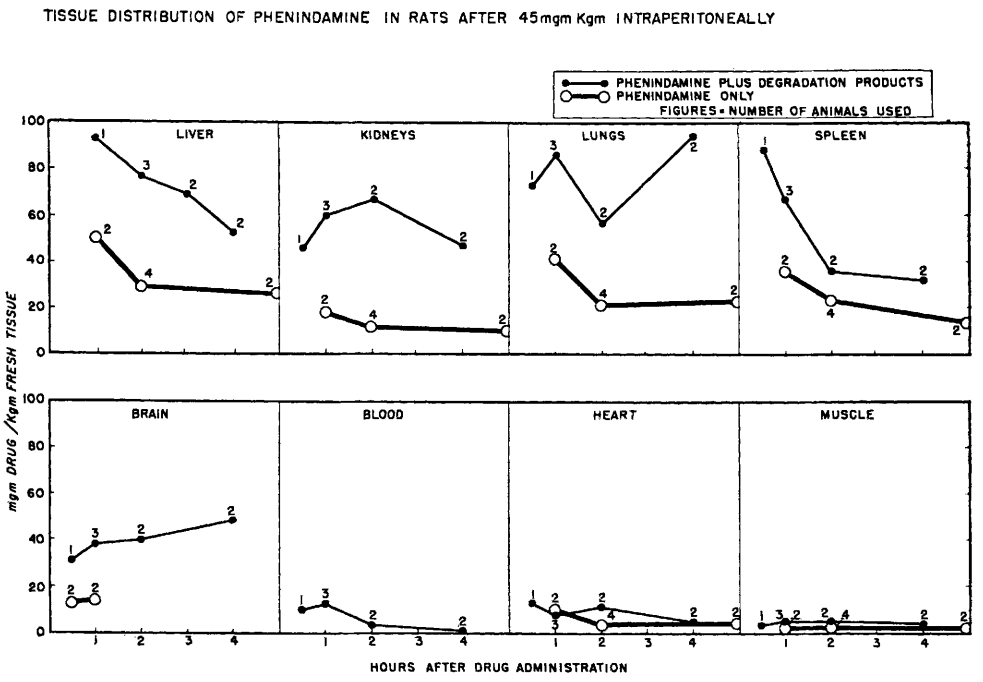

FIG. 2.

phenindamine 45 mg/kg intraperitoneally, the animals were sacrificed at definite time intervals. Various organs were removed and analyzed for phenindamine content in the manner previously described. A series of tissue levels were also determined omitting the quadruple buffer wash of the benzene extract in the procedure. The tissue levels of phenindamine obtained with and without the buffer wash are summarized in Fig. 2. The two methods differ considerably as to the absolute amount of phenindamine present in each tissue, but show a similar trend as to the ability of various organs to concentrate the drug. Since the unwashed benzene extract measures the compound plus its degradation products which also react with methyl orange, whereas the washed benzene extract essentially measures only phenindamine, it is quite apparent that phenindamine is very rapidly metabolized in the body. The compound appears to leave the blood rapidly and localize in tissues. High concentrations of the phenindamine were found in the lungs, liver, kidneys and spleen. Appreciable concentrations were also present in brain, whereas the levels in the blood, heart and muscle were barely detectable. Appreciable levels of phenindamine were still demonstrable in the lungs even after 5 hours. These findings appear to support the findings of Lehmann on guinea pigs, that phenindamine has a longer duration of action than most antihistamines(1). However, extensive storage of the compound does not seem to occur. These conclusions are based on our experimental findings made on 4 rats which received 20 mg/kg of phenindamine twice daily for 4 consecutive days. On analyzing the tissues of the animals for phenindamine 24 hours after the last administration, no phenindamine could be detected. These results with phenindamine resemble our previous results with Pyribenzamine(5), although the latter appears to disappear more rapidly from the body.

Excretion of phenindamine. The kidney appears to play only a minor role in the excretion of phenindamine. In total 24 hour urine samples collected from 3 humans (males) following administration of 50 mg orally, less than 2% of the total dosage was recovered as phenindamine. Likewise, in 2 rats, less than 2% of the total dose was re-

covered in the urine 24 hours after giving 100 mg/kg, intragastrically. Since less than 10% of the dose was recovered concomitantly from the gastrointestinal tract and its contents, it is concluded that for the most part phenindamine is completely metabolized in the body.

Summary. Phenindamine (Thephorin) was determined in biologic material with a high degree of specificity by a modification of the Brodie methyl orange technic. Negligible amounts of phenindamine can be recovered in the urine of humans following oral administration. In studies on rats the compound appears to be rapidly absorbed from the gastrointestinal tract. The compound rapidly leaves the blood and concentrates in tissues. High concentrations of the drug were found in the lungs, liver, kidneys and brain. Extensive accumulation of phenindamine did not occur after repeated administration of the compound twice daily for 4 consecutive days. It is concluded that the compound is metabolized to a considerable extent in the body since less than 12 per cent of the total dose of phenindamine could be recovered unchanged in the urine and feces 24 hours following oral administration.

We are grateful to the American Allergy Foundation and the Division of Research Grants, National Institutes of Health, U. S. Public Health Service, for generously supporting our studies, to Dr. E. L. Sevringhaus of Hoffman-La Roche, Inc., for supplying the samples of Thephorin, and to Dr. P. K. Smith for his comments and advice.

Received May 22, 1950. P.S.E.B.M., 1950, v74.

Relationships of Vital Capacity and Ventilatory Measurements to Physical Fitness in Patients with Cardio-Respiratory Diseases.* (17920)

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Previous studies have shown a direct relationship between physical fitness scores of working capacity and the availability of oxygen to the tissues in an heterogeneous group of ambulatory patients with cardio-respiratory diseases(1). The clinical evaluation of disability in these patients varied from virtually none to complete and total disability, with the majority comprising the intermediate ranges. Physical fitness was scored from an integration of the most reproducible characteristics of circulatory and pulmonary performance during standardized exercise. The first of these was the duration of time that moderate exercise on a treadmill ergometer could be tolerated (up to an arbitrary maximum of 10 min.). The other criteria were the mean respiratory efficiency (volumes % of oxygen absorbed per unit volume of air ventilated) during exercise, and the total number of heart beats for the first 3 minutes of recovery(2). Others have found significant relationships of the vital capacity, the maximum breathing capacity and the breathing reserve to the awareness of dyspnea in patients with pulmonary diseases(3, I-III). In view of these considerations, it becomes important to ascertain how well vital capacity, and various measures of ventilatory performance commonly used in evaluating

* Aided by grant from National Institute of Health, Contract H-222.

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1. Bruce, R. A., Lovejoy, F. W., Jr., Yu, P. N. G., and McDowell, M., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 212.

2. Bruce, R. A., Lovejoy, F. W., Jr., Yu, P. N. G., and McDowell, M., Pearson, R., *Science*, 1949, v110, 442.