at a dosage of 0.1 mg/kg of hydrochlorothiazide and 1 mg/kg of chlorothiazide.

Both chlorothiazide and hydrochlorothiazide are secreted by renal tubules, being cleared at rates in excess of glomerular filtration rate. Both compounds, thus, will be removed rapidly from blood of normal dogs following a single intravenous dose. The mechanism whereby both chlorothiazide and hydrochlorothiazide are excreted in excess of glomerular filtration presumably involves that utilized by penicillin, p-aminohippurate and certain other organic acids, inasmuch as it is suppressed by administration of probenecid, an agent known to depress selectively renal tubular secretion of certain organic acids without affecting electrolyte excretion and without interference in renal transport of numerous other substances(7).

Summary. Hydrochlorothiazide is a potent saluretic agent. It resembles chlorothiazide qualitatively, but is several times more active, intravenously and orally, in the dog.

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A Metabolite of 1-Hydrazinophthalazine (Hydralazine).* (24657)

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The antihypertensive drug 1-hydrazinophthalazine (hydralazine, apresoline) has been shown to interfere in the process of biological acetylation of sulfanilamide(1). The mechanism for this interference appeared to be competition between substrate and drug for the active acetyl group. It was further shown that free hydralazine disappeared from pigeon liver extracts when all components of the acetylation system were present. These facts suggested the possibility that 1-acetyl-2-phthalazyl hydrazine (acetyl hydralazine) should be formed as a conjugate of hydralazine. We here report the identification of this material as a product of hydralazine metabolism *in vitro* and *in vivo*.

Materials and methods. In vitro incubations and extractions. An adult pigeon was killed by decapitation and its liver rapidly dissected out and placed in cold Robinson's solution(2) containing 20 mg of glucose per 100 ml. Ten grams of liver slices were prepared with the Stadie-Riggs microtome. The slices were divided into 2 equal portions and each washed twice with Robinson's solution and finally suspended in 10 ml of the solution containing 200 μmoles of sodium acetate and in one case 100 μmoles of hydralazine. The latter materials had been previously made up in a small portion of Robinson's solution and neutralized before incroporation into the suspending medium. The mixtures were incubated with shaking at 37° for 4 hours with air as the gas phase. The contents of the vessels were then ground in a Potter-Elvehjem type homogenizer with a close-fitting teflon pestle. Three drops of glacial acetic acid were added to each homogenate and the mixtures deproteinized by allowing them to stand for 5 minutes in a boiling water bath. After centrifuging, the pH of each supernatant was adjusted to 9-10 with

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dilute NaOH solution and extracted twice with 10 ml portions of ethyl acetate. The extracts were combined. An adult rat was killed by decapitation, its liver dissected out, and a 50% liver homogenate prepared by grinding the tissue in 0.14 M KCl. To each of two 5 ml portions of the homogenate were added the following, all except the buffer having been previously neutralized: 400 μmoles of sodium acetate, 20 μmoles of sodium citrate, 10 μmoles of cysteine hydrochloride, 5 μmoles of coenzyme A, 10 μmoles of adenosine triphosphate, and 2.5 ml of tris (hydroxymethyl) aminomethane buffer at pH 8.0. To one of the vessels 100 μmoles of neutralized hydralazine hydrochloride were added and to the other an equal volume of water. The final volume of the mixtures was 12.5 ml in both cases. After a 2-hour incubation period, under the conditions described above, the mixtures were deproteinized and the supernatants extracted as above. *In vivo experiment.* A female guinea pig weighing 1.1 kg was injected subcutaneously with 10 mg of hydralazine hydrochloride and placed in a metabolism cage for urine collection. After an 8.5-hour interval, another 10-mg dose of hydralazine hydrochloride was administered. Twenty-six hours after the first injection, the collected urine was adjusted to a pH of 9-10 and extracted as the tissue supernatants above. A similar collection of control urine had been made previously. *Synthesis of acetylhydralazine.* One gram of hydralazine · HCl was dissolved in 10 ml of water and the solution made basic with dilute KOH. It was extracted twice with 10-ml portions of chloroform and the chloroform solution dried over anhydrous Na_2SO_4. The chloroform was removed by evaporating the solution to dryness in a round-bottom flask. The residue was taken up in 30 ml of benzene. Twenty milliliters of pyridine were added followed by 12 ml of acetic anhydride. This mixture was refluxed for 6 hours. After evaporation to dryness *in vacuo* at 100°, a brown, oily residue remained. This was dissolved in 95% ethanol, and water was added to incipient cloudiness. After standing in the cold for a short time, crystals formed. These were collected by centrifugation, recrystallized twice from ethanol-water mixtures, and once from 95% ethanol. After air-drying, the white crystals melted sharply at 178°. Microanalysis†: C, 59.41%; H, 5.11%; N, 27.66%: Calc'd: C, 59.41%; H, 4.95%; N, 27.72%. *Chromatography.* All paper chromatography was performed on strips 1″ in width of Whatman#1 filter paper by the ascending technic.

Results. When samples of ethyl acetate extracts of liver incubation mixtures or guinea pig urine were chromatographed in a number of solvents, brilliant white fluorescent zones were observed on strips under ultraviolet lamp in extracts from incubation mixtures containing hydralazine and from urine from the guinea pig given hydralazine. No such zone was found on strips prepared from control extracts. That this material was not unchanged hydralazine was shown by comparing chromatograms of hydralazine. Hydralazine does not fluoresce in the ultraviolet, but exhibits a dark brown absorbing zone. When chromatograms containing hydralazine were sprayed with a 0.1% alcoholic solution of picryl chloride and then exposed to ammonia vapor, a dark red-brown zone appeared. No such zone was apparent on chromatograms of the ethyl acetate extracts. Hydralazine showed different R_f values from those given by the white fluorescent material.

Extracts gave a chromatographic zone identical to that of synthetic acetyl hydralazine. Table I shows R_f values in a number of solvent systems of synthetic acetyl hydralazine and the zones obtained from either the guinea pig urine, the pigeon liver slices, or the fortified rat liver homogenate.

The white fluorescent material formed by pigeon liver from hydralazine and that present in guinea pig urine after administration of hydralazine was purified by successive paper chromatography in 22% isopropyl alcohol, water, n-butanol: 0.6 N NH_4OH (6:1), and 10 N acetic acid. After each chromatographic separation, the white fluorescent zones were cut from the strip, taking care to take only the most dense section and discarding the leading and trailing edges. The excised zone was cut into small pieces and eluted with 95%

† Schwarzkopf Microanalytical Lab., Woodside, N. Y.

ethanol. The ethanol extract was then used for the succeeding chromatograms. Fig. 1 shows the absorption spectra of the materials and of authentic acetyl hydralazine as determined on the Beckman DU spectrophotometer. The extract from the rat liver homogenate was carried through the same purification procedure; however it still exhibited appreciable absorption below 230 mμ. But in the range 240-250 mμ it gave an identical spectrum.

In none of the extracts was free hydralazine detectable. When the extracts were evaporated to dryness, taken up in 1 N HCl and allowed to hydrolyze on the steam bath for an hour, it was possible to demonstrate free hydralazine.

TABLE I. R_f Values of Acetyl Hydralazine and White Fluorescent Zone from Ethyl Acetate Extracts.

Solvent	Acetyl hydralazine	White fluorescent zone from: Guinea pig urine	Pigeon liver	Rat liver
22% isopropyl alcohol	.74	.72	.73	.72
60% *idem*	.85	.86	.84	
Water	.64	.62	.59	.60
2.5 N acetic acid	.81	.81	.82	
10 N *idem*	.86	.85	.89	.89
n-Butanol: 0.6 N NH_4OH (6:1)	.90	.88	.88	.89
20% $CHCl_3$: 80% n-butanol (water saturated)	.00	.00	.00	.00

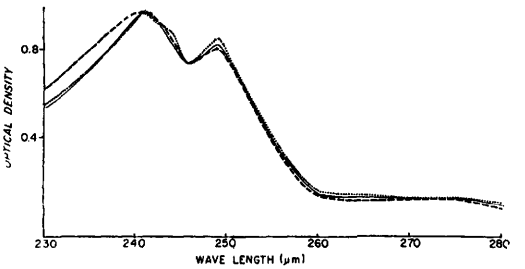

FIG. 1. Ultraviolet absorption spectra of authentic acetyl hydralazine (solid line), white fluorescent material from pigeon liver (dotted line), and guinea pig urine (dashed line).

From the above experiments, and from our previous(1) observations that for appreciable hydralazine disappearance from tissue extracts all the components of the metabolic acetylation system must be present, it seems evident that acetyl hydralazine is a major metabolic product of hydralazine.

Summary. 1-Acetyl-2-phthalazyl hydrazine (acetylhydralazine) has been identified as a metabolic product of hydralazine in the rat, guinea pig, and pigeon.

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Action of Biogenic Amines, Amine Oxidase Inhibitors, and Other Agents on Chromatophores of Squid, *Loligo pealii*.* (24658)

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Regulation of smooth muscle-chromatophore system in the squid involves neurohumoral mechanisms which bear certain points of resemblance to smooth muscle systems in higher forms. Chromatophores of cephalopod molluscs have been extensively studied since the original work of Phisalix(1), but primarily for their own intrinsic value. Particular attention has been given to central neural controls. The purpose of the present experiments was to study local control of smooth muscle-chromatophore system in the squid, *Loligo pealii*. Particularly the action on this system, of biogenic amines known to be effec-

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