that these steroids are not responsible for the phenomenon noted.

On the basis of Rosenblum's(6) observation that exogenous pitressin lowers EST, one would expect that removal of an endogenous source, *i.e.*, neurohypophysectomy, should give a rise in EST. Since the opposite effect occurred, the question arises whether endogenous and exogenous pitressin influences EST by the same mechanism. The endogenous hormone may have a more complex regulatory effect, perhaps involving other endocrine factors, in contrast to the exogenous one, which may directly stimulate some portion of the central nervous system.

*Summary.* The effects of total hypophysectomy, adenohypophysectomy and neurohypophysectomy on EST were compared in rats. 1. Hypophysectomy and adenohypophysectomy severely lowered EST and reduced body and adrenal weight. 2. Neurohypophysectomy, although reducing EST had less effect on threshold than the other operations, without significantly altering body weight and adrenal size.

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## Disturbance in Porphyrin Metabolism Caused by Feeding Diethyl 1, 4-dihydro-2, 4, 6-trimethylpyridine-3, 5-dicarboxylate. (24705)

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In previous studies on fluorescence of forestomach in mice various fluorescent and nonfluorescent substances were fed to determine stainability of mucus in the forestomach. When mice that received diets containing diethyl 1, 4-dihydro-2, 4, 6-trimethylpyridine-3, 5-dicarboxylate (hereafter called "collidine derivative") were sacrificed for examination and photography of fluorescence in beam of near ultraviolet light, the gall bladder and liver appeared to be red fluorescent. Since the substance itself was intensely blue fluorescent, the red fluorescent bile and livers were thought to be a reflection of an increased porphyrin excretion. This investigation involves the study of the nature of this type of altered porphyrin metabolism. Dose relationship has been studied and types and amounts of porphyrins involved were determined.

*Materials and methods.* Fifty-one Strain A male mice, weighing 20 to 25 g, were fed a diet containing 2.5 g of collidine derivative/45 g ground food. Animals were sacrificed daily and liver and kidneys removed immediately for examination in beam of near ultraviolet light. One-gram samples of liver, cut so as to include the gall bladder and upper portions of biliary tree, were ground in a mortar with ethyl acetate-glacial acetic acid (4/1 v/v). Extractions were continued until red fluorescence could no longer be detected in the supernate. Total extract was fractionated according to the method of Schwartz

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TABLE I. Hepatic Porphyrin Concentrations in Mice Treated with Collidine Derivative. μg porphyrin/g liver.

| Day on drug | No. of animals | Protoporphyrin Range | Protoporphyrin Avg | Coproporphyrin Range | Coproporphyrin Avg | Protoporphyrin/coproporphyrin |
|---|---|---|---|---|---|---|
| 0 | 7 | | | 0 – trace | | |
| 1 | 4 | 48 – 160 | 111 | trace – 15.5 | 9.2 | 11.2 |
| 2 | 4 | 58.5– 226 | 142.6 | .36– 30 | 13.5 | 10.5 |
| 3 | 4 | 143 – 332 | 241 | 6.8 – 28.4 | 18.2 | 13.2 |
| 4 | 7 | 130 – 388 | 262 | 27.6 – 38.8 | 30.0 | 8.7 |
| 5 | 5 | 166 – 398 | 277.5 | 9.3 – 64 | 34.4 | 8.05 |
| 6 | 4 | 312 – 775 | 630 | 11.4 – 85.2 | 45.5 | 13.7 |
| 7 | 4 | 428 – 822 | 635 | 22.4 –108 | 55.3 | 11.4 |
| 8 | 4 | 550 –1056 | 850 | 43 –160 | 88.0 | 9.75 |
| 9 | 4 | 840 –1150 | 947 | 76 –177 | 123.5 | 7.7 |
| 10 | 4 | 860 –1160 | 976.2 | 128 –237 | 158.0 | 6.2 |
| | | | | | Ratio avg | 10.04 |

and Wikoff(1). Fluorometric analvses were carried out on aliquot samples employing a modified Lumetron Fluorometer. Additional identification of protoporphyrin fraction was carried out by study of visible absorption spectrum. The various porphyrin fractions were also analyzed by unidimensional paper chromatography technic of Erickson(2). Urinary porphyrin studies were carried out on albino male guinea pigs, fed 150 mg of collidine derivative/day in the ground food. No restrictions were placed on amount of lettuce animals received. The 24 hour urine samples, collected from animals while housed in special metabolism cages, were analyzed for porphobilinogen according to procedure of Watson and Schwartz(3) and for coproporphyrin employing 5 ml method of Schwartz, Zieve, and Watson(4). Urinary uroporphyrin was demonstrated by $Al_2O_3$ chromatography technic of Schwartz *et al.*(5).

*Results.* Data presented in Table I indicate that the oral administration of collidine derivative to mice, results in progressive increase in hepatic proto- and coproporphyrin concentrations. The ratio of protoporphyrin to coproporphyrin was approximately 10 to 1. Examination of Hematoxalin and Eosin stained sections of mouse liver from treated animals revealed presence of large numbers of dark red-brown particles in parenchymal cells, Kupfer cells, and bile ducts in portal areas. Frozen sections of treated liver, covered with a drop of glycerin and examined immediately under fluorescence microscope showed no characteristic red fluorescence. However, approximately 4 minutes after preparation, red fluorescence became evident at periphery of numerous small particles scattered throughout the unstained preparation. This fluorescence increased in intensity until it filled the microscopic field.

Examination of kidneys of mice after treatment with this agent revealed no red fluoresence until seventh day. Then, the kidney became orange-red fluorescent. When cut surfaces of sections were stained, a thin line of intense red fluorescence could be observed midway between renal cortex and pelvis. Extraction of this material in fluorescent renal tissue revealed that it consisted of large quantities of protoporphyrin and much smaller amounts of uroporphyrin.

Serum of mice treated 24 hours with collidine derivative became pink fluorescent. Characterization of porphyrins responsible for this has not been accomplished.

Studies of urinary coproporphyrin and porphobilinogen in the guinea pig treated with collidine derivative are presented in Table II. Maintenance of high urinary coproporphyrin levels appears dependent upon continued administration of the drug. When treatment with this agent was discontinued, the 24-hour urinary coproporphyrin output fell to trace quantities within 4 days. Urinary porphobilinogen, present in low concentrations on several occasions when the animal was receiving the agent does not appear to follow any characteristic excretory pattern.

TABLE II. Urinary Coproporphyrin and Porphobilinogen Excretion by Guinea Pig (740 g) Treated with Collidine Derivative, 150 mg/Day.

| Day on drug | μg/24 hr Copro-porphyrin | Porpho-bilinogen |
|---|---|---|
| 1 | 0 | — |
| 2 | 0 | — |
| 3 | Trace | — |
| 4 | 49 | + |
| 5 | 110 | — |
| 6 | 158 | — |
| 7 | 147 | Trace |
| 8 | 150 | " |
| 9 | 138 | — |
| 10* | 146 | Trace |
| 11 | 75.5 | — |
| 13 | 46.5 | — |
| 14 | 27.2 | — |
| 15 | Trace | — |

* Collidine derivative stopped.

*Discussion.* Abnormalities in porphyrin metabolism characterized by high levels of protoporphyrin have been discussed by Schultz and Schwartz(6). Presence of both proto- and coproporphyrin was reported by these investigators in their study of experimental chloroma. In various chloromas, protoporphyrin was approximately 20 times more concentrated than coproporphyrin. Studies of allyliso propylacetylcarbamide (Sedormid) porphyria in rabbits by Schmid and Schwartz (7) revealed increases in hepatic uro-, copro-, and protoporphyrin. Protoporphyrin was approximately 6 times more abundant than coproporphyrin in fresh livers of animals treated with this agent. Analysis of urinary porphyrins excreted, indicated a marked increase in uroporphyrin and a smaller increase in coproporphyrin. No urinary protoporphyrin could be demonstrated. Urinary porphobilinogen roughly paralleled excretion of uroporphyrin. Similar results have been reported by Case(8) for urinary porphyrin levels in rats treated with Sedormid. Illumination of viscera of rabbits treated with Sedormid(7) in beam of near ultraviolet light revealed intense red fluorescence produced by protoporphyrin in gall bladder, bile ducts, and duodenal region adjacent to orifice of common bile duct.

Though collidine derivative and Sedormid (7) are of unrelated chemical structure, the disturbance of porphyrin metabolism produced by oral administration of these agents is similar in that hepatic proto- and coproporphyrin excretion by liver, are increased in both instances. The intense red fluorescence of gall bladder, biliary tree, and duodenal region is due in each case to a marked increase in protoporphyrin.

There appear to be differences in urinary porphyrin excretion in the 2 types of chemically-induced porphyria. In animals treated with Sedormid(7) for 7 days, very large quantities of uroporphyrin and smaller amounts of coproporphyrin were present in urine. Animals receiving collidine derivative one week, excreted large amounts of coproporphyrin and smaller amounts of uroporphyrin in their urine. Urinary porphobilinogen excretion also differed. In Sedormid(7) porphyria, excretion of porphobilinogen roughly paralleled urinary uroporphyrin levels, while in porphyria induced by collidine derivative no characteristic excretory pattern for porphobilinogen could be observed.

*Summary.* Oral administration of collidine derivative (Diethyl 1, 4-dihydro-2, 4, 6-trimethylpyridine-3, 5-dicarboxylate) to mice produces a disturbance in porphyrin metabolism characterized by marked increases in hepatic proto- and coproporphyrin. Red fluorescence of kidneys of animals treated with this agent for 7 days is associated with great increase in renal protoporphyrin and smaller increase in renal uroporphyrin. In the guinea pig, oral administration of collidine derivative produced high levels of urinary coproporphyrin and trace amounts of urinary porphobilinogen.

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## Effect of Hypothalamic Lesions on Tryptophan Peroxidase of the Liver.* (24706)

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Independent studies show that activity of tryptophan peroxidase in the liver of rats is under partial control of the pituitary-adrenal system(1,2,3,4). Enhanced enzyme activity follows administration of "non-specific" compounds which presumably release adrenocorticotrophin (ACTH). Augmented enzyme activity is also produced by ACTH or cortical steroids(1,2,3,4). Following adrenalectomy, non-specific agents do not produce increased enzyme activity. Tryptophan, on the other hand, augments enzyme activity by a mechanism independent of adrenal cortical hormones (1). Since release of ACTH appears to be regulated, at least in part, by the hypothalamus(5,6), it appeared of interest to determine response of tryptophan peroxidase to non-specific agents, using animals with hypothalamic lesions which block secretion of ACTH. The agent chosen was histidine since it has been more thoroughly investigated than other agents(1). The results indicate that hypothalamic lesions produce a decrease in response of tryptophan peroxidase to histidine.

*Methods.* Adult male rats (Long-Evans) weighing 200-300 g, fed a synthetic diet(7), were used. The Krieg stereotaxic instrument was used to produce hypothalamic lesions, designed to interrupt the supraoptico-hypophysial tract in median eminence of tuber cinereum(5,6). Only rats with successful interruptions of this tract as reflected by severe diabetes insipidus with water intake in excess of 100 ml, 24 hours following induction of lesions were used. In 6 instances, location of lesions was determined by serial sections of the hypothalamic area, and the lesions lay in median eminence of the tuber cinereum. Earlier studies show that such rats subjected to acute stress give no evidence of ACTH release(5,8). Since no discernible difference in response to histidine could be detected 2, 4 and 5 days post-operatively, the combined data were pooled. Seven days intervened between hypophysectomy and histidine injection. Five hours prior to nercopsy, half the rats in respective groups received intraperitoneal (I.P.) injections of 2 mM 1-histidine-HCl; the remaining animals were uninjected and served as controls. The rats were anesthetized with Nembutal (5 mg/100 g, IP) and decapitated. Immediately afterwards the liver was removed, chilled, and a weighed portion was used in tryptophan peroxidase activity determination(1). The method used is an indirect one and depends on kynurenine production after incubation of liver homogenates with added tryptophan as substrate, activity being expressed as number of $\mu$M of kynurenine/gram dry weight liver/hour. In some cases and immediately following removal of liver, the left adrenal was removed and analyzed for ascorbic acid concentration(5). The relevant data are summarized in Tables I and II.

*Results. Effect of histidine on ACTH release.* It has been assumed that histidine produces its effect on tryptophan peroxidase by stimulating secretion of ACTH, which, in turn, brings about release of cortical steroids

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