metabolism, and to study the pharmacological mechanism of action of DIMPEA at the synaptic terminals of dopaminergic fibres[19].

Résume. L'effet d'une injection de 100 mg/kg de 3,4-diméthoxyphényléthylamine (DIMPEA) sur l'excrétion urinaire des catécholamines a été étudié chez le rat en cage métabolique. Il est montré que le DIMPEA produit une augmentation significative de l'excrétion urinaire d'acide homovanillique (HVA), le principal métabolite de la dopamine. Le mécanisme d'action plus précis a été étudié chez le chien et il est proposé que le DIMPEA produit une accélération du «turnover» de la dopamine par une augmentation de synthèse de l'amine, elle-même consécutive à un bloc au niveau des terminaisons dopaminergiques.

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An Effect of Diet on Homovanillic Acid Excretion

Recently, VON STUDNITZ and NYMAN[1] reported that a chromatographic spot corresponding to dimethoxyphenethylamine (DMPEA) disappeared from the urine of 9 subjects when they were fed a glucose diet flavored with citric acid. VON STUDNITZ interpreted these data to signify that DMPEA was present in the diet although he did not analyze food material for the presence of this compound. While exclusion diets have frequently been used to determine whether a compound is derived from exogenous sources, it is questionable whether the disappearance of a compound from urine during this kind of regimen is evidence that the compound is of dietary origin. For example, GOODMAN[2] has shown that the withdrawal of food for 24 h results in a sharp decrease in the excretion of vanillylmandelic acid (VMA) in human subjects, although this compound is known to be a metabolic product of noradrenaline.

In order to investigate some of the complex relationships involved in dietary studies, a study of the effect of a glucose diet on a known metabolic product was undertaken. It has previously been shown that homovanillic acid (HVA) is the major metabolite of dopamine. In rats about 60% of administered dopamine C^{14} is converted to HVA[3]. VON STUDNITZ et al.[4] studied the effects of a glucose diet in humans on the excretion of this acid as well as a number of other acids. He found that HVA was excreted during the glucose diet, but he did not report whether there was a change in its concentration. In the present study rats were studied rather than humans inasmuch as complete dietary control is much more readily obtained in laboratory animals.

Procedure and Results. Two 250 g Holzman rats were placed together in each of 2 metabolic cages and offered an ad libitum diet of Wayne Lab Blox. The rats were permitted to accommodate to the laboratory and the cage for several days before the experiment was started. Water was not restricted. At the end of this time, food was removed, the cages were cleaned and a urine sample was collected for 24 h, from each group of rats, in a vessel containing 5 cm^3 of 0.1 *N* HCl. At the end of the 24 h urine collection the rats were offered a diet consisting of 5% glucose solution in water. After 24 h of this diet urine collection was started and continued for the next 24 h. The entire 24 h sample was assayed by the method of ARMSTRONG et al.[5], except that the extract was chromatographed on a 1 dimensional strip, ascending. The area corresponding to HVA was then eluted and chromatographed on a second 1 dimensional strip, also ascending. The solvents were respectively: isopropanol-ammonia-water, 8:1:1, and benzene-isopropanol-water, 2:2:1. The area corresponding to HVA on the strips from the pre-glucose urines was compared with those obtained from urines collected after glucose diet. All strips showed a strong area corresponding to HVA before glucose diet. Post glucose strips did not contain detectable amounts of HVA, however.

In order to preclude the possibility that HVA was present in the Wayne rat food used in this study, 6 pellets of rat food were subjected to analysis for HVA. This amount is in excess of the amount fed to each group of rats during the 24 h period. The pellets were mashed in 0.1 *N* NaOH, until a fine slurry was produced, and then filtered. The solution was extracted and chromatographed as described for the urine samples[5]. No spot corresponding to HVA was detectable.

6 additional pellets were also treated by a procedure devised for the extraction of phenolic acids from foodstuff[6]. The extract obtained by this procedure was chromatographed in 2 consecutive 1 dimensional systems, as above. After the second chromatographic separation, the area corresponding to HVA was eluted with methanol, rechromatographed on 1 dimensional thin layer plates coated with silica gel and developed in butanol-ethyl acetate-ammonia, 3:1:1, ascending. Again no spot corresponding to HVA could be detected in the rat food.

A third group of rats was also subjected to a glucose diet as described for the other 2 groups, and urine was collected and processed as detailed for the other urine samples. The area corresponding to HVA was eluted after

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the second chromatographic separation and subjected to thin layer chromatography as described for the food. A spot corresponding to HVA was found in the pre-glucose urine but was not detectable in the post glucose urine.

An O-acetyl methyl ester derivative[7] was prepared from HVA added to urine and treated by the above extraction and chromatographic procedures. From chromatography of this derivative in an F & M Biomedical Model 400 gas chromatograph it was determined that the recovery of HVA was about 60% of the added material.

It was determined, therefore, that HVA excretion is markedly reduced upon glucose feeding, although HVA is known to be a metabolic product of dopamine and is believed to be of endogenous origin.

Discussion. The importance of diet in influencing chemicals found in the body fluids and particularly in urine has long been recognized. However, the possible complexity of this relationship has often been ignored. It has frequently been assumed that, if a product disappears from the urine when potential food sources have been removed, this product was in fact contained in the food. In this study we have demonstrated that the concentration of a known metabolic product, HVA, can be strikingly reduced by dietary modification even though its presence in the food cannot be demonstrated by 2 different extraction techniques. The apparent decrement in HVA in the urine could have occurred because of an increase in conjugation in the presence of a large increase in glucose[8], a decrease in available precursors or a shift in urinary pH resulting in diminished renal clearance[9]. 1 or more of these factors may also be involved in the findings of Von Studnitz in regard to DMPEA[1]. These alternative possibilities appear to offer at least as good an explanation as that DMPEA is ingested in food.

Zusammenfassung. Reine Glukose-Diät führt zur Abnahme der Ausscheidung von Homovanillinsäure im Rattenharn. Die Abnahme trat auch dann ein, wenn keinerlei präformierte Homovanillinsäure im Rattenfutter nachgewiesen werden konnte.

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Effect of Locally Applied Acetylcholine on the Embryonic Cardiac Action Potential

Acetylcholine (ACh) is known to cause an increase in P_K in mammalian sinoatrial (SA) and atrial fibres[1] and has therefore been employed in attempts to analyse the components of the cardiac action potential. By use of locally applied ACh, atrial and perinodal action potentials have been shown to consist of an initial, fast component (spike) followed by a prolonged, slow component (plateau), whereas potentials from SA and AV (atrioventricular) nodal cells contain only the slow component[2]. These findings provide the basis for considering the development of the cardiac action potential as consisting of 2 functional parts: a fast process representing the P_{Na} system[1,3] and a slow process involving, in part, a decrease in P_K[1,4]. Other studies have considered the dual nature of the amphibian cardiac action potential from several points of view[5].

The present study was undertaken, first, to reveal the presence or absence of the 2-component system in transmembrane potentials recorded from embryonic chick heart cells and, second, to relate the findings to the development of electrical activity in the embryonic heart.

The procedure for preparing the embryonic chick hearts (14–19 days) for microelectrode analysis is described in detail elsewhere[6]. Acetylcholine (60 μg/ml) is applied to the preparation by local ejection of a modified Tyrode solution through a micropipette (tip diameter 50 μ) situated very close to the recording microelectrode. This technique was adapted from similar studies of the adult mammalian heart[2] and enables simultaneous intracellular recording during local applications of acetylcholine.

The Figure (A, B, C, D) shows the effect of this procedure on 4 types of cardiac action potentials. In Figure A, the atrial action potential is shortened in duration, but the peak potential is unaltered or slightly decreased. Figure B shows the response of a transmembrane action potential from the atrial margin of the AV ring, in which acetylcholine produces a marked decrease in peak potential as well as a shortened action potential. Although not shown in the Figure, ventricular cells do not noticeably respond to the local applications of ACh. However, the action potential duration of cells in the ventricular portion of the AV valve is still somewhat sensitive to acetylcholine (Figure C). It is of interest to note the progressive delay in the response of the valve cell to the driving stimulus (not visible on record). A progressive delay in AV transmission due to acetylcholine is known to occur in the embryonic chick heart[7]. Thus, the response shown in Figure C is most probably caused by ACh, as it affected cells of both the AV ring and AV valve. The depolarizing

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