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Summary

Vobasine, a monomeric indole alkaloid of novel spectral type, and voacryptine, a monomeric 5-methoxyindole derivative, have been isolated from the bark of *Voacanga africana* Stapf.

'Active Ammonia'

In the course of our investigations on the possible active forms of ammonia, we studied the amination of α-keto acids by rat liver preparations. Liver from freshly sacrificed rats was washed with saline, minced and homogenized in saline with a Potter-Elvehjem type homogenizer. The incubation was carried out in 0·1 M pH 7·0 phosphate buffer. Each vessel received 20 μmol. of sodium pyrophosphate and 5 μmol. of malonic acid. The following additions were made at the level of twenty μmol. of each: adenosine triphosphate (ATP), ammonium phosphate, α-keto glutaric acid (or pyruvic acid) and adenosine phosphoramide (AMP-NH_2). The total volume was 3·2 ml. At the end of 2–3 h at 38°C trichloroacetic acid was added to a final concentration of 5%. The incubates were centrifuged and analyzed for amino acid by the ninhydrin method. The keto acid left was estimated by the method of FRIEDEMANN and HAUGEN[1]. The results are presented in Table I.

Table I
Amino acid formation in rat liver homogenates

No	Additions	Keto acid disappeared μmol	Amino acid formed μmol
1	None (tissue alone) . . .	None added	3·90
2	α-ketoglutaric acid (K.G.)	0·0	5·10
3	K.G plus ammonium phosphate	0·0	11·85
4	K.G plus ammonia plus ATP	20·0	28·65
5	K.G plus ATP	17·82	23·65
6	K.G plus AMP-NH_2 . . .	20·0	24·45

The results presented in Table II were obtained by elimination of each component from a complete medium consisting of liver homogenate, phosphate buffer, α-keto glutaric acid, pyrophosphate, malonic acid and adenosine phosphoramide. Using pyruvic acid in the place of α-keto glutaric acid, similar results were obtained.

[1] T. E. FRIEDEMANN and G. E. HAUGEN, J. biol. Chem. *147*, 415 (1943).

Table II
Relative importance of the components of the medium

Medium of Incubation	Keto acid disappeared μmol	Amino acid formed μmol
Complete medium	20·0	25·6
Minus malonic acid	19·67	32·7
Minus pyrophosphate . . .	19·67	26·8
Minus α-keto glutaric acid .	****	10·8
Minus liver homogenate . .	0·0	0·0
Minus AMP-NH_2	10·35	13·4

These data show that adenosine phosphoramide was as effective as a mixture of ATP and ammonia. ATP alone was quite active and this might be due to the endogenous formation of ammonia in the liver homogenate. Further experiments are in progress to test whether the role of AMP-NH_2 is confined to amino acid synthesis alone or is important in other amination (e.g. biosynthesis of glucosamine) and amidation (e.g. glutamine biosynthesis) reactions also. Further experimental details will be reported elsewhere. While these experiments were in progress, similar results were reported using preparations of *Mycobacterium*[2].

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Zusammenfassung

Es wurde gefunden, dass Adenosinophosphoamidat (Na) die Synthese von Keto-Säuren aus Rattenleberhomogenaten anregt. Diese Verbindung kommt in ihrer Wirkung einer Mischung von Adenosintriphosphat und Ammonium gleich. Wir beabsichtigen, die Amino- und die Amido-Reaktionen der Reinverbindungen zu untersuchen, um festzustellen, ob die physiologisch wirksame Form des Ammoniums aus Adenosinphosphoamidat besteht.

[2] N. KATUNUMA, Arch. Biochem. Biophys. *76*, 547 (1958).

The Effect of Substance P on the Amount of 5-Hydroxytryptamine in the Gut

It has been shown in a previous work that substance *P* restored peristalsis when injected intraluminally into the isolated guinea-pig ileum in which the peristaltic reflex was abolished by fatigue, by external or internal application of 5-hydroxytryptamine, or by lowering the temperature of the bath[1]. On the other hand, substance *P* when acting on the outside of the isolated guinea-pig ileum produced a block of the peristaltic reflex[2]. 5-Hydroxytrypt-

[1] D. BELESLIN and V. VARAGIĆ, Brit. J. Pharmacol. *13*, 321 (1958).
[2] D. BELESLIN and V. VARAGIĆ, J. Pharm. Pharmacol. *11*, 99 (1959).

Table I
The effect of substance P on the amount of 5-HT in the ileum, stomach and spleen of the rat

Number of experiments	ILEUM (ng 5-HT/g)		P	STOMACH (ng 5-HT/g)		P	SPLEEN (ng 5-HT/g)		P
	Controls	Substance P 100 U/kg		Controls	Substance P 100 U/kg		Controls	Substance P 100 U/kg	
1	1130	1574		1126	2086		1871	2307	
2	1303	2742		1598	1860		1000	1498	
3	1215	1670		1056	1452		714	1918	
4	754	1336		1553	1558		857	1428	
5	720	2218		988	1684		1124	615	
6	826	1578		891	2072		1076	1198	
7	340	1800		1919	2374		1810	1995	
8	902	1460		711	1743		1082	1018	
9	1480	1572		951	2198		972	1047	
10				1694	2308		705	1869	
Mean ± St. Error	964 ± 118·1	1773 ± 146·1	< 0·01	1249 ± 119·9	1933 ± 96·2	< 0·01	1121 ± 128·4	1489 ± 191·8	< 0·1

amine affected peristalsis in a similar way, i.e. it stimulated peristalsis when acting on the mucous membrane of the intestine[3] and it depressed or abolished peristaltic reflex when acting on the outside of the isolated intestine[4,5]. It was suggested in a previous work that substance *P* may act through another active substance[1]. Considering the similarity with which substance *P* and 5-HT act on the peristaltic reflex, the present experiments were undertaken in order to see whether substance *P* can alter the 5-HT content of the gut.

In the isolated guinea-pig ileum of 15–20 cm long, the substance *P* (60–80 units) was injected intraluminally and was allowed to act for 20–35 min. The control preparation was injected with saline. Both control and test preparation were prepared according to a modified Trendelenburg's technique which is described in detail in a previous paper[1]. The substance *P* was then washed out and the ileum was extracted with 80% acetone according to the method of CORREALE[6]. The extracts were assayed on the isolated rat fundus[7] in the presence of atropine (2×10^{-8} g/ml) and antazoline (10^{-6} g/ml).

In another series of experiments on rats, substance *P* was injected intraperitoneally in doses ranging from 60–120 units/kg. The injections of substance *P* were repeated in three successive days and on the fourth day the animals were sacrificed. From each animal the stomach, the spleen and a piece of ileum 20 cm long were taken and extracted with aceton. The extracts were assayed against 5-hydroxytryptamine on the isolated rat fundus in the presence of atropine (2×10^{-8} g/ml) and antazoline (10^{-6} g/ml).

The results are presented in the Tables I and II.

It may be seen that substance *P* increased the amount of 5-hydroxytryptamine both in the isolated guinea-pig ileum and in the rat ileum. The same was true for the rat stomach. On the other hand, no change in the 5-hydroxytryptamine content of the rat spleen was observed.

Table II
The effect of substance P on the amount of 5-HT in the isolated guinea-pig ileum

Number of experiments	Controls (ng 5-HT/g)	Substance P 60–80 Units Intraluminally (ng 5-HT/g)	P
1	350	640	
2	627	928	
3	551	1064	
4	339	604	
5	641	961	
6	414	686	
7	665	830	
8	467	691	
9	417	2618	
10	562	1115	
Mean ± St. Error	503 ± 35·1	1014 ± 154·2	< 0·01

STACEY and SULLIVAN[8] have found that an increase in the tryptophan content of the diet of mice and rats resulted in a rise in the amount of 5-HT in the small intestine. Sterilization of the gut by streptomycin and chlortetracycline had a similar effect, probably by preventing bacterial metabolism of dietary tryptophan. The present experiments show that intraluminal or intraperitoneal injection of substance *P* may also increase the amount of 5-hydroxytryptamine in the stomach and in the ileum. The exact mechanism of this effect of substance *P* cannot be accounted for by the present experiments.

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Résumé

L'injection intrapéritonéale de la substance *P* (60 à 120 U/kg) chez le rat provoque une augmentation mar-

[3] E. BÜLBRING and R. C. Y. LIN, J. Physiol. *138*, 12P (1957).
[4] H. W. KOSTERLITZ and J. A. ROBINSON, J. Physiol. *136*, 249 (1957).
[5] K. H. GINZEL, J. Physiol. *137*, 62P (1957).
[6] P. CORREALE, J. Neurochemistry *2*, 201 (1958).
[7] J. R. VANE, Brit. J. Pharmacol. *12*, 334 (1957).
[8] R. S. STACEY and T. J. SULLIVAN, J. Physiol. *137*, 63P (1957).

quée de 5-HT dans la paroi de l'estomac et dans l'iléon. Dans la rate de cet animal, on n'y observe pas d'augmentation de 5-HT.

L'administration intraluminale de la substance *P* (60–80 U) dans l'iléon isolé du cobaye, augmente aussi sensiblement la quantité de 5-HT.

On the Excretion of 17-Ketosteroids in Guinea Pigs

Several investigations have revealed that the adrenocortical activity in guinea pigs is relatively high. They therefore excrete a number of 17-ketosteroids (17-KS) in the urine. PERON *et al.*[1] isolated the following 17-KS from *male* guinea pig urine:

3α-hydroxyetiocholan-17-one, 3α-hydroxy-$\Delta^{9(11)}$-etiocholen-17-one, 3α-hydroxyetiocholan-11,17-dione, 3α-,11β-dihydroxyetiocholan-17-one,3α-hydroxy-$\Delta^{9(11)}$-androsten-17-one, 3α-hydroxyandrostan-11,17-dione, 3α,11β-dihydroxyandrostan-17-one.

They were all 3α-compounds, of which the final identification was made by infrared analysis.

In our experiments, which will be reported in detail elsewhere, we isolated a *3β-hydroxy-17-ketosteroid* from the urine of normal untreated *female* guinea pigs. This has not previously been reported, under similar experimental conditions. Moreover, we confirmed the findings of PERON *et al.*[1].

The urine of 10 female guinea pigs was collected daily for 6 days. After β-glucuronidase hydrolysis and subsequent cold acid hydrolysis at pH 0·5 with continuous ether extraction (for 48 h), the 17-KS containing extracts were chromatographed on alumina by gradient elution technique[2]. We obtained 7–8 Zimmermann-positive fractions. As is shown in Table I, fraction II was the greatest. It amounted to 44·9% of the total Zimmermann chromogens eluted and contained a 17-KS, which showed the same running rate as crystalline *epiandrosterone* on the paper chromatogram (chromatographic system used: propylene glycol/ligroin). In the mixed chromatogram of both the isolated substance and pure epiandrosterone, no separation occurred.

The sulphuric acid spectrum of the unknown revealed maximal optical density at 310 mμ. No increased absorption was apparent at 405 mμ, which is characteristic for dehydroepiandrosterone. The paper chromatographic properties of epiandrosterone and dehydroepiandrosterone are not much different. Both steroids have almost the same running rate in the system propylene glycol/ligroin.

The 3β-configuration of the isolated steroid was demonstrated by digitonin precipitation of the purified fraction II. Less than 10% of it appeared in the α-fraction, while the bulk of Zimmermann positive material was measured in the β-fraction. The infrared spectrum of the β-fraction was identical with that of crystalline epiandrosterone. The described substance could be isolated repeatedly in different series of experiments.

The question as to the nature of the 17-KS conjugates appearing in the urine of guinea pigs seemed to be of particular interest. In order to investigate the mode of conjugation of 17-KS in guinea pigs, we followed the procedure of CREPY *et al.*[3] given for the extraction and separation of steroid conjugates in human urine.

A 6-day-urine specimen collected from 10 female guinea pigs was adjusted to pH 11, followed by the addition of sodium chloride until a saturation of approximately 10% was achieved. The urine was then extracted twice with the same volume of n-butanol. The extract so obtained was chromatographed on alumina as reported by CREPY *et al.*[3]. In this manner it was separated into 3 Zimmermann-positive fractions, the second of which (eluted with 10% aqueous butanol) corresponded to 17-KS sulphates, the third of which (eluted with 15% aqueous (0·1 N NH_4OH) butanol) to 17-KS glucuronides (Table II). Subjected to high voltage paper electrophoresis the 10 % aqueous-butanol fraction revealed the mobility of Rb = 0·86, which is typical for 17-KS sulphates[4]. After cold acid hydrolysis (at pH 0·5) and simultaneous continuous ether extraction (for 48 h) the paper chromatographic evaluation of the above fraction disclosed 3α-hydroxyetiocholan-11,17-dione and higher polar 17-KS (probably 3α,11β-dihydroxy etiocholan-17-one and 3α,11β-dihydroxyandrostan-17-one). The glucuronide fraction (eluted with 15% aqueous

Table II

10 female guinea pigs-urine collection for 6 days. Extraction of conjugates with butanol at pH 11; chromatography of the extract on alumina (according to CREPY[3]).

Eluate	17-Ketosteroids (% of total amount eluted)	Glucuronic Acid
Butanol; 2% aqueous butanol	30–40%	–
6% aqueous butanol	–	–
10% aqueous butanol	40–50%	–
15% aqueous (0·1 N NH_4OH) butanol . . .	3–6%	1·8 mg

[1] F. G. PERON and R. I. DORFMAN, J. biol. Chem. *223*, 877 (1956).

[2] T. K. LAKSHMANAN and S. LIEBERMAN, Arch. Biochem. Biophys. *53*, 258 (1954). – W. STAIB and W. SCHILD, Klin. Wschr. *36*, 166 (1958).

[3] O. CREPY, M. F. JAYLE, and F. MESLIN, Acta endocrinol. *24*, 233 (1957).

[4] H. PELZER and W. STAIB, Clin. chim. Acta *2*, 407 (1957).

Table I

10 female guinea pigs-urine collection for 6 days. Gradient elution chromatography on alumina after β-glucuronidase hydrolysis and subsequent cold acid hydrolysis (at pH 0·5) with continuous ether extraction (for 48 h).

Total 17-Ketosteroids	Relative distribution of the 17-KS; recorded in % of the total 17-KS-excretion						
15·9 mg	Fraction I 10·3	Fraction II 44·9	Fraction III 5·3	Fraction IV 9·1	Fraction V 10·9	Fraction VI 14·3	Fraction VII 5·2