Some years ago Dickinson, Spencer and Gorham (1) described an acute respiratory syndrome in cattle induced by feeding 0.5 gm./kg. body weight of tryptophan. The pulmonary lesions reported included oedema formation in the interstices and the alveolar air spaces. The production of a substance acting on smooth muscle, especially on vascular smooth muscle in these experiments, seems a possibility worth consideration. We therefore decided to test the hypothesis that the most obvious candidates, tryptamine or 5-hydroxytryptamine (5-HT), might be formed from tryptophan by the microorganisms in the rumen. From the outset decarboxylation of tryptophan seemed quite likely, whereas the introduction of a hydroxyl group was rather improbable in view of the overall reducing properties of ruminal fluid.

**Methods**

Ruminal fluid, essentially free of solids, was collected by stomach tube from healthy adult cows and brought to the laboratory in a prewarmed Dewar bottle. Hay, on which the cows were feeding, was cut with scissors and thoroughly moistened with 0.1 M NaHCO₃ solution in a kitchen blender. About 1/4 of this mash was mixed with 3/4 of ruminal fluid and 500 ml. of the mixture was incubated in an airtight glass jar within a shaking water bath at 38°—39 °C. Connexions for gas and bicarbonate solution were made through holes in the lid which also carried a pH-electrode. The gas space of the system was initially flushed with 5 percent CO₂ in nitrogen and after the vessel was sealed the pressure was kept at 3 cm. H₂O by means of a wash bottle in the gas outlet connexion. Rate of gas production and changes of pH were monitored (3). When gas and acid production had become steady (usually after one hour), L-tryptophan (1 mg./ml. in most experiments) was added as dry powder. The pH was kept between 6.4 and 7.0 by adding 1 M NaHCO₃ solution when necessary. When the metabolic activity (rate of gas production) declined, cellobiose (1 gm. at a time) was added as dry powder. For addition
of solids the seal had to be broken for a few seconds. The incubation lasted for several hours (see table). Different controls were run: (a) In three experiments the control sample was identical with the experimental sample, but was immediately cooled in ice and processed instead of being incubated. (b) In two experiments the control sample was identical with the experimental sample and was incubated for the same time but was boiled before or after adding the mash. (c) In one experiment the control was treated as the experimental sample but received no tryptophan.

At the end of the incubation period the 500 ml. samples were rapidly cooled in an ice bath and sonicated at 100 watt for 5 min. (Branson Sonifier B-12). The total fluid was acidified with 1000 ml. of 0.1 n HCl, vigorously shaken and filtered through gauze. It was then brought to pH 9 by adding solid Na₂CO₃, saturated with NaCl and extracted with 500 ml. n-butanol by shaking for 10 min. The phases were separated by centrifugation and the butanol (green) phase was washed twice with an equal volume of 0.1 M Na-bicarbonate-carbonate buffer at pH 10. Ten parts of butanol extract were mixed with 15 parts of heptane and the mixture was shaken with 2.5 parts of 0.1 n HCl. The water phase was collected and dried at 50° under reduced pressure in a thin film evaporator. The residue was taken into 5 ml of water and assayed biologically or chromatographed on thin layer plates.

For the bioassay the rat stomach fundus preparation (2) was used. The fundus region was dissected and stored in Krebs solution at 4°C overnight. A strip of about 2 by 10 mm. was mounted in an organ bath containing Krebs solution saturated with 5% CO₂/95% O₂, and its tension development was recorded with a Grass force displacement transducer on a Grass polygraph. 1/50 dilutions of the extracts were compared in a three-point assay with different concentrations of 5-HT (as creatinine-SO₄) (Fig. 1). The comparison was made with 5-HT rather than with tryptamine, because at elevated concentrations of tryptamine the relaxation of the strip was slow. The equipotent ratio on a weight per volume basis of tryptamine-HCl/5-HT-creatinine-SO₄ measured at 50 percent maximum effect for tryptamine was 95 ± 33 (mean of 4 experiments ± SEM). From this the tryptamine concentration in the extracts was calculated. Due to the large standard error of the equipotent ratio the absolute amounts of tryptamine given might be wrong by a factor of two and serve only as a guide to the correct order of magnitude. The response of the preparation was inhibited by adding 10⁻⁷ gm./ml. of methysergide to the bath. This helped to demonstrate that the response to the extracts was due to a substance affecting the 5-HT-receptor and/or the tryptamine receptor. 10⁻⁷ g./ml. methysergide was able to abolish tryptamine responses equivalent to the response to the extracts. The small residue of contraction after methysergide was subtracted from the response obtained before inhibition in order to correct for active agents other than 5-HT or tryptamine in the extracts.

The extracts were examined by thin layer chromatography on silica gel-CaSO₄ plates in two different solvents, namely propanol/methylacetate/7 M ammonia (45/35/20) (solvent a), or acetone/7 M ammonia (100/1) (solvent b). The spots were localized by their characteristic fluorescence in UV light (at 254 or 360 nm) or by fluorescence quenching on plates containing a fluorescent indicator (MERCK). In both solvents the 5-HT-creatinine complex dissociates, creatinine moving more slowly than 5-HT. Therefore the complex could be used as standard for 5-HT. On storing the plates for several weeks in air the spots turned brown or yellow. In some experiments a large amount of extract was chromatographed on a whole plate, the silica gel at the tryptamine
level was collected and eluted with 0.1 n HCl. The eluate was rechromatographed or its UV-spectrum was recorded with a Beckman DB instrument connected to a W + W 3002 recorder.

L-tryptophan, tryptamine-HCl and 5-HT-creatinine-sulfate were obtained from FLUKA (Buchs, Switzerland). Silica gel was the product of MACHEREY and NAGEL (Düren, Germany), ready-made thin layer plates with a fluorescent indicator were from MERCK. Glucose was purchased from BDH Chemicals (England). All other substances and reagents were analytical grade products of MERCK or FLUKA.

Results

The extraction procedure used in the present experiments separates organic bases and uncharged lipid-soluble compounds from other substances in the sample. Provided that there was no loss, a 100-fold concentration was achieved by the extraction. Figure 1 and Table 1 show that the extracts of

![Graph](https://via.placeholder.com/150)

Fig. 1. Example of tension development of the rat gastric fundus preparation under the influence of ruminal extract. Recording of tension versus time. 0.1 ml. of the extract was added to 5 ml. of bathing fluid. Comparison with 5-HT-creatinine-S04. Above: extract after incubation with 1 mgm./ml. L-tryptophan, below: control (incubation of boiled ruminal fluid with 1 mgm./ml. L-tryptophan), both from Expt. 5. Notice complete abolition of response by $10^{-7}$ gm./ml. methysergide
samples incubated for several hours with 50 or 500 mgm. L-tryptophan per 500 ml. contained a substance activating the gastric fundus preparation. The effect was almost completely abolished by 10^{-7} gm./ml. methysergide, a specific inhibitor of 5-HT and tryptamine action. In the table the activity of the extracts is expressed as concentrations (w/v) of 5-HT-creatinine-sulfate. It may be seen that roughly ten times the activity of the controls was found in extracts incubated with L-tryptophan under favourable conditions. The controls ensure (a) that the active substance was not present as a contaminant in the tryptophan used, (b) that it was not produced by the extraction steps, (c) that it was not present initially in large amounts in ruminal fluid or hay, (d) that incubation was necessary to produce it, (e) that it was not formed without addition of tryptophan, and (f) that some heat-labile factor, present in the ruminal fluid but not in the hay, was required for its production. Presumably the factor destroyed by heat is an enzyme, most probably of microbial origin. The pharmacological evidence suggests that the active substance is 5-HT or tryptamine.

**Table 1**

Activity of extracts containing the active material from 500 ml. ruminal fluid in 5 ml. aqueous solution on the rat gastric fundus preparation, expressed as gm./ml. of 5-hydroxytryptamine-creatinine-sulfate (5-HTC)

<table>
<thead>
<tr>
<th>Expt. number</th>
<th>Incubation time (hrs)</th>
<th>Viable sample 5 - HTC (gm/ml x 10^{-6})</th>
<th>Control sample 5 - HTC (gm/ml x 10^{-6})</th>
<th>Tryptophan concentration (mgm/500 ml)</th>
<th>Type of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0.22</td>
<td>0</td>
<td>50</td>
<td>not incubated , with tryptophan</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2.4</td>
<td>0.042</td>
<td>500</td>
<td>not incubated , with tryptophan</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0.48</td>
<td>0.041</td>
<td>500</td>
<td>not incubated , with tryptophan</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1.0</td>
<td>0.0875</td>
<td>500</td>
<td>incubated , without tryptophan</td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td>3.5</td>
<td>0.52</td>
<td>500</td>
<td>boiled with mash , with tryptophan</td>
</tr>
<tr>
<td>6</td>
<td>3.5</td>
<td>0.975</td>
<td>0.05</td>
<td>500</td>
<td>boiled before mash , with tryptophan</td>
</tr>
<tr>
<td>mean of expts. 2 - 4</td>
<td>1.67 *</td>
<td>0.148 *</td>
<td></td>
<td></td>
<td>p = 0.03 for the difference</td>
</tr>
</tbody>
</table>

(mean of expts. 2—4 should read 2—6)

Thin layer chromatography in two systems, separating satisfactorily tryptophan, tryptamine and 5-HT, showed that tryptamine was present in detectable amounts in extracts from tryptophan supplemented samples after incubation in a viable state but not in any of the controls. 5-HT was not detected in experimental samples or controls. The possibility cannot be ruled out that some 5-HT was present but that the amount contained in 0.1 ml. extract used for chromatography fell below detectability. Fig. 2 A shows a two-dimensional chromatogram in the two solvents of an extract from the viable sample of experiment 5. Extract and standards were run simultaneously in the same tank. The control (boiled) is omitted in the figure; it did not show a spot corresponding to tryptamine. Fig. 2 B presents a one-dimensional chromatogram of experiment 4. There was a clearly visible spot with an Rf-value corresponding to tryptamine in the extract from the tryptophan sample,
Fig. 2. A. Two-dimensional chromatogram of extract from Expt. 5. Direction I: propanol/methylacetate/ammonia (solvent a); direction II: acetone/ammonia (solvent b). Standards dissolved in 2 n acetic acid; 5-HT = 5-hydroxytryptamine-creatinine-SO₄. Left panel: extract from viable fluid, right panel: standards. Both plates run in the same tank simultaneously. Silica gel-CaSO₄ plates, 0.3 mm thick. Photograph at 360 nm. Standard spot of 5-HT is drawn into the photograph because it was not clearly visible on the print. Fluorescence immediately after drying the plate: yellow for tryptamine, blue for tryptophan. Control (not shown) without spot corresponding to tryptamine. — B. One-dimensional chromatogram of extract from Expt. 4 on a Merck-plate. Solvent a: propanol/methylacetate/ammonia. 1: tryptophan standard (4 and 2 μ-litres of 1 % solution); 2: tryptamine-HCl standards (2 and 4 μ-litres of 1 % solution). E: redchromatographed extract from experimental sample, E₄: control (no tryptophan added to ruminal fluid), E₅: extract from experimental sample. Amount of extract used: 0.1 ml. Silica gel-CaSO₄ plates with fluorescent indicator. Photograph in visible light after standing. Pencil lines around clearly visible spots. S: Start, F: Front

whereas no spot could be detected at the same level in the extract from the control, incubated without tryptophan.

Fig. 3 shows UV absorption spectra from Experiment 5. The region corresponding to tryptamine was eluted from thin layer chromatograms with 0.1 n HCl. Curve A is from the viable sample and curve B from the control, boiled before incubation, both measured against 0.1 n HCl. Curve C represents the difference between the sample eluate and the control eluate. The sample eluate shows the characteristic absorption spectrum common to tryptamine and tryptophan. The control shows little, if any, material with this absorption spectrum. For comparison the spectrum of pure tryptamine was recorded simultaneously.
Fig. 3. UV-spectra of eluates from thin layer chromatograms of Expt. 5. Two-fifths of the total extract (5 ml.) were chromatographed and the tryptamine region was eluted with 0.1 n HCl. The eluate from the experimental sample was diluted 1:3 before the measurement, the control sample was used undiluted. — Curve A: experimental sample, curve B: control (boiled before incubation), curve C: differential spectrum between A and B. Molar extinction for tryptamine in 0.1 n HCl at 278 nm. : 4720

Discussion

The present results demonstrate that viable ruminal contents from cows produce a pharmacologically active substance when incubated for several hours under anaerobic conditions with 1 mgm. per ml. of L-tryptophan. The substance was identified as tryptamine since (a) its effect on the rat stomach fundus preparation is abolished by methysergide; (b) the extract contains a substance with the Rf-value of tryptamine on thin layer chromatograms in two different solvents and this substance, after isolation, shows the UV absorption spectrum of tryptamine. The possibility, however unlikely, that small amounts of 5-HT were produced in addition cannot be ruled out.

Assuming that 5-HT-creatinine-sulphate is about 95 times as potent per mgm. as tryptamine-HCl in the bioassay used, the amount of tryptamine produced during the average incubation time of 4 hr. by 500 ml. of ruminal fluid can be calculated. The mean value amounts to 3.15 μ-moles. From the extinction at 278 nm. in Fig. 3 a production of 5.6 μ-moles of tryptamine is obtained. The bioassay for this particular experiment gave a value of 8.5 μ-
moles. The agreement between the two methods is satisfactory in view of the inevitable loss of material in eluting the chromatograms and the error incurred in the bioassay.

Since 2.45 m-moles of tryptophan were added the yield is only about 1/800. No attempt was made to assess either the time or concentration dependence of the observed decarboxylation of tryptophan by ruminal fluid. We do not know whether under the experimental conditions chemical equilibrium was reached or whether the concentration of tryptamine found was due to a steady state, tryptamine being formed and degraded at equal rates at that concentration. We can state with certainty, however, that the small yield was not due to tryptophan being exhausted by other processes (for instance by deamination or by incorporation into proteins) because in all experiments tryptophan was still detectable by chromatography at the end of the incubation.

In the ruminant neither the rate of absorption of tryptamine from the gastrointestinal tract nor its fate after having reached the circulation is known. Furthermore the question remains open as to whether under in vivo conditions decarboxylation of tryptophan in the rumen is equally effective as in vitro. It would be rash, therefore, to conclude from the present finding that excess dietary tryptophan necessarily leads to gastrointestinal, pulmonary or other systemic ill-effects by way of tryptamine formation in the rumen. Nevertheless, it might be worth considering this possibility in pathological states related to particular types of feeding not easily explained otherwise.

Summary

Evidence is presented to the effect that viable ruminal fluid from cows incubated under anaerobic conditions in vitro is able to decarboxylate added L-tryptophan (1 mg./ml.) partly to tryptamine. The possible pathophysiological bearing of the production of this biologically active amine in the rumen is briefly discussed.

Zusammenfassung

Die Bildung von Tryptamin aus Tryptophan durch Pansensaft in vitro

Pansensaft vermag in vitro unter anaeroben Verhältnissen L-Tryptophan (1 mg/ml) partiell zu Tryptamin zu dekarboxylieren. Die pathophysiologische Bedeutung der Bildung dieses biologisch aktiven Amins im Pansen wird diskutiert.

Résumé

Production de tryptamine à partir du tryptophane par le liquide du rumen in vitro

On démontre que le liquide du rumen des vaches, incubé dans des conditions anaérobies, in vitro, est capable de décarboxylérer partiellement du L-tryptophane ajouté (1 mg/ml) en tryptamine. On discute brièvement l'aspect physiopathologique de la production de cette amine biologiquement active dans le rumen.

Resumen

Producción de triptamina a partir de triptófano por el líquido ruminal in vitro

Se presenta evidencia en el sentido de que el líquido ruminal viable de vacas, incubado bajo condiciones anaerobias in vitro, es capaz de decarboxilar
parcialmente el L-triptófano añadido (1 mg./ml.) a triptamina. De forma breve se discute la situación fisiopatológica posible de la producción de esta amina biológicamente activa en la panza.

References

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