to the time of contact between catalase and filtrate (Fig. 1 *b*) and to the amount of filtrate used (Fig. 1 *c*). A spontaneous decline of the inhibitory power of the preparation was observed after 24 h standing in the cold (Fig. 1 *d*). The activity, however, was restored when the ovomucoid was re-boiled.

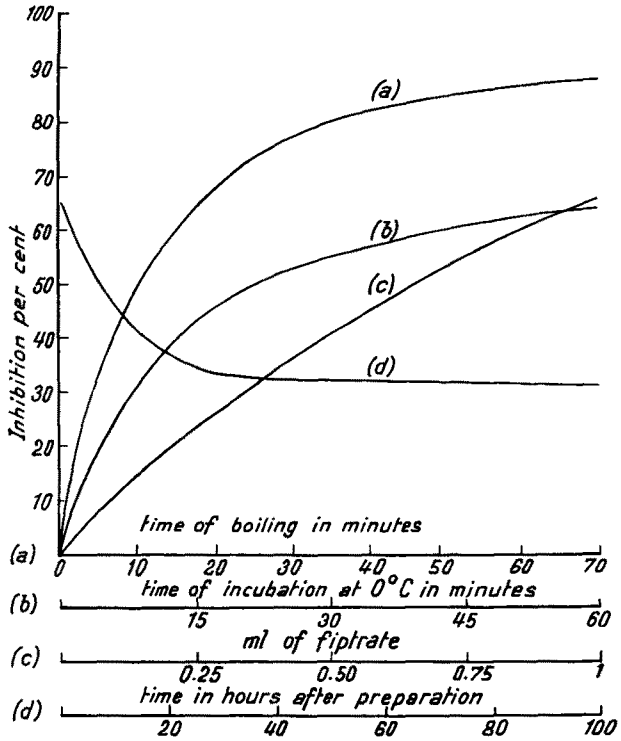

Fig. 1.–Anticatalase activity of ovomucoid. Effect of the time of boiling *a*, of the time of incubation *b*, of amount of filtrate *c* on inhibitory power; spontaneous decline of the inhibitory power *d*.

Furthermore lyophilized or ethanol-precipitated ovomucoid, when dissolved in distilled water, proved to be inactive. Again, activity was restored by boiling. The same loss of activity occurred as a result of a 48 h dialysis of ovomucoid against distilled water at the room temperature. Boiling of the dialyzed ovomucoid after addition of any one of the following salts: KCl, $CaCl_2$, $MgCl_2$, NaCl(0·065 *M*) restored the activity. The sulphates of the same cations were ineffective in bringing about the activity.

From the above it appears that ovomucoid can acquire by means of boiling a new molecular configuration which seems to be responsible for its anticatalase activity. Denaturing agents other than boiling thus far assayed (X-rays, UV-rays, urea) have proven to be ineffective in causing anticatalase activity of ovomucoid to appear.

The new configuration appears to be unstable as it can easily and spontaneously revert to the apparently more stable, inactive one. On the other hand, boiling of ovomucoid causes a loss of its anti-tryptic power.

Furthermore we have found that both trypsin and chymotrypsin attack the "active" ovomucoid much more readily than the "inactive". This suggests that the "activation" of ovomucoid may be considered as a process of reversible denaturation.

These observations raise the question whether the anticatalase activity of kochsaft of tumors may not be due to a glycoprotein.

	mg N/ml in the supernatant after 1 h digestion at 35°C of	
	"active" ovomucoid	"inactive" ovomucoid
Trypsin	0·53	0·12
Chymotrypsin	0·34	0·25

We are indebted to Prof. A. Monroy, Institute of Comparative Anatomy, Palermo, for advice and suggestions.

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Biological Department, Centro Tumori, Palermo, May 11, 1954.

Riassunto

L'ovomucoide acquista spiccata attività anticatalasica dopo ebollizione. L'ovomucoide attivo dopo liofilizzazione o precipitazione alcoolica diviene inattivo; l'attività viene ripristinata con nuova ebollizione. L'ovomucoide attivo anche dopo dialisi perde l'attività che viene riacquistata con l'aggiunta di sali e successiva ebollizione. L'attività anticatalasica è dovuta ad una nuova configurazione instabile dell'ovomucoide.

Observations on the Metabolism of Endogenous 5-Hydroxytryptamine (Enteramine) in the Rat

The organism of the rat contains about 125 μg of 5-hydroxytryptamine(5-HT) per kilogram of body weight, in terms of free base: 34 μg are present in the blood, 11 μg in the spleen and 80 μg in the gastrointestinal mucosa[1].

In normal rat urine an indole compound was found which is chromatographically indistinguishable from 5-hydroxyindoleacetic acid (5-HIAA).

The following solvents and developing agents were used:

(a) solvents: n-butanol saturated with N HCl; n-butanol-acetic acid-water mixture (4:1:5); n-butanol-monomethylamine 25–30% (8:3); amylalcohol-pyridine-water (2:2:1).

(b) developing agents: 2% alcohol solution of p-dimethylaminobenzaldehyde + HCl vapours; Heinrich and Schuler's N.N.C.D. reagent in 0·1 NHCl; diazotized p-nitroaniline + ammonia vapours[2].

From 155 adult rats weighing 26·5 kg, 775 ml of urine were collected in a 12 h period, i.e. about 59 ml/kg/24 h. At the semiquantitative estimation the urine showed a 5-HIAA content of approximately 1·34 μg per milliliter, viz. of 81·4 μg/kg/24 h or 3·4 μg/kg/h.

It is highly probable that urinary 5-HIAA originates from the oxidative deamination of 5-HT. This signifies that 3·1 μg/kg of endogenous 5-HT are excreted every hour as 5-HIAA (1 mg 5-HIAA = 0·92 mg 5-HT).

On the basis of researches on the fate of exogenous 5-HT administered by parenteral route, we may suppose that only one third of endogenous 5-HT can be recovered from urine as 5-HIAA. The remaining two thirds seem to undergo more profound breakdown processes, possibly involving the rupture of the indole ring[3].

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[2] V. Erspamer and G. Boretti, Arch. int. Pharmacodyn. *88*, 296 (1950).

[3] V. Erspamer, forthcoming publication.

We can therefore conclude that about 9–9·5 μg of 5-HT are destroyed every hour per kilogram of body weight (= 216–236 μg/kg/24 h).

The above quantitative data may be taken as a basis for some considerations.

(1) The metabolism of endogenous 5-HT is very intense: blood 5-HT could be renewed every 3½ h, intestine 5-HT every 8–9 h, and the total 5-HT of the organism every 12–14 h.

This metabolic rhythm rules out the possibility of platelet 5-HT, i.e. virtually of blood 5-HT, being synthetized and incorporated by the platelets at the site of their formation[1], since the life span of the rat platelets is reported to be approximately 3 to 4 days[2]. No evidence exists in favour of a synthesis of 5-HT in the mature circulating platelets[3], which, on the contrary, seem to be capable of absorbing 5-HT from plasma[4].

The quantitative data now available on the metabolism of endogenous 5-HT strongly support our opinion that the site of formation of 5-HT in the organism is the enterochromaffin cell system.

(2) The writer and his collaborators[5] have shown that in hydrated rats the minimum antidiuretic dose of 5-HT is as small as 4 μg/kg, by subcutaneous route.

The failure by CORCORAN *et al.*[6] to confirm these results must presumably be ascribed to inadequacy in their experimental procedure and to the insufficient number of experimental animals they have used.

If we keep in mind that in the rat organism 9–9·5 μg of endogenous 5-HT are inactivated every hour, and that inactivation requires of necessity the presence of free substance in the plasma, we must conclude that the antidiuretic action of 5-HT complies with all the necessary conditions to consider it as the "physiological" action of the substance.

Rat serum contains an antidiuretic factor of non-pituitary origin, called "stable antidiuretic substance"[7] This factor has been recently identified as 5-HT[8].

It is enough to inject subcutaneously 1 ml of serum per 100 g of rat to reduce significantly the urine flow. But again the amount of 5-HT present in this milliliter is not greater (1·05 μg) than that which is released every hour into the circulating plasma by the enterochromaffin cells.

No biological action, besides the antidiuretic one, has been so far obtained with "physiological" doses of 5-HT, i.e. with substance doses of the order of those which occur and are metabolized in the organism.

From all the above it seems to us that evidence is growing in favour of the hypothesis which considers 5-HT as a hormone regulating the function of the kidney, presumably through an interference in the control of intrarenal hemodynamics.

(3) We believe that quantitative estimation of urinary 5-HIAA will become in the near future the method of preference for investigating the rate of 5-HT metabolism under normal and pathological conditions.

It is hoped that the results of these studies will substantially contribute to a more complete understanding of the biological meaning of 5-HT.

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Zusammenfassung

Der Rattenurin enthält 1,34 μg 5-Hydroxyindolylessigsäure je Kubikzentimeter (= 81,4 μg/kg/24 h). Daraus ist zu berechnen, dass in der Ratte täglich 216–236 μg/kg 5-Hydroxytryptamin metabolisiert werden. Dies bedeutet, dass das Blutplättchen-5-HT alle 3–4 h und das Gesamt-5-HT des Organismus alle 12–14 h erneuert werden wird.

Die erhaltenen quantitativen Daten sind hauptsächlich im Hinblick auf die fragliche Bildungsstelle des 5-HT und die biologische Bedeutung der Substanz selber wichtig.

Zur Wirkung von Narkotin auf den Hustenreflex und auf die Bronchialmuskulatur

Auf der Suche nach Hustenmitteln, die frei von zentralen Nebenwirkungen (Euphorie, Suchtgefahr) sein sollten, prüften wir u.a. auch Narkotin. Dieses in beträchtlicher Menge im Opium vorkommende Alkaloid enthält im Gegensatz zu Morphin und Codein einen Isochinolinring ähnlich wie Papaverin. Soweit ältere Untersuchungen über Narkotin vorliegen, erweisen sie eine kurzdauernde Erregung der Atmung hinsichtlich Frequenz und Volumen[1] sowie eine Erschlaffung glatter Muskelfasern, zum Beispiel des Darms[2]. Jedenfalls fehlt nach den vorliegenden Angaben dem Narkotin eine nennenswerte analgetische oder narkotische Wirkung, woraus hervorgeht, dass der Name den Wirkungstypus keineswegs trifft. Narkotin hat therapeutisch bis jetzt keine Bedeutung erlangt.

Die Auslösung von Husten erfolgte an den mit Numal-Roche (45–55 mg/kg i.p.) narkotisierten Katzen durch mechanische Reizung der Schleimhaut der Luftröhre. Zu diesem Zweck führten wir in Intervallen von 2 bis 5 min. eine Hühnerfeder durch einen kleinen Einschnitt in der Luftröhre bis zur Teilung in die beiden Hauptbronchien ein. Die Hustenstösse registrierten wir mit einer Marey-Kapsel, die über einen Seitenarm mit einer im Pharynx knapp oberhalb des Kehlkopfes befindlichen Kanüle verbunden war. Als Vergleichssubstanz wählten wir Codein, dessen hustenstillende Wirkung im Experiment und in der Praxis unbestritten ist.

Unsere bisherigen Versuche ergaben das überraschende Ergebnis, dass dem Narkotin eine ausgesprochen hustenstillende Wirkung zukommt. Narkotin verminderte gelegentlich schon in Dosen von 0,1 mg/kg das Ausmass der Hustenstösse. In Dosen von 0,5 und 1,0 mg/kg unterdrückte Narkotin meistens den Hustenreflex (s. Abb. 1).

Narkotin erwies sich in unseren Versuchen mindestens so stark hustenstillend wirksam wie Codein, das den

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