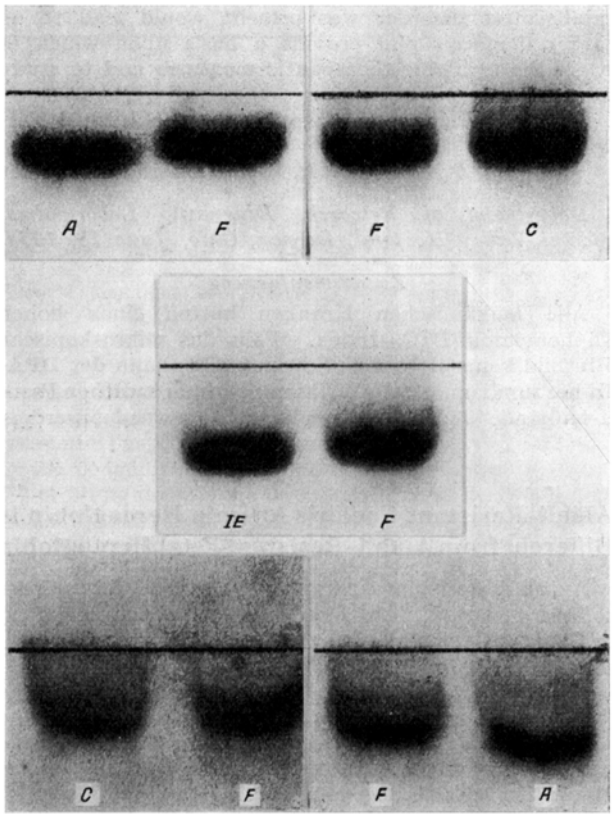
Now we have been able to show that paper electrophoresis provides evidence of a different behaviour for fetal and COOLEY's anemia Hbs.

After repeated trials, we have used the following technique which is very similar to that described by REYNAUD[1]. The FLYNN and DE MAYO electrophoretic apparatus was used filled up with sodium veronal buffer (pH 9). Two Hb spots were applied on each the 6 cm strips of WHATMAN filter paper n.1, by means of PEROSA's gadget[2]. After 12 h electric current flow (120 W), immediate fixation was accomplished with an alcohol-ether mixture.



Examples of different electrophoresis runs. *A* = Adult normal Hb; *F* = Fetal Hb (umbilical cord blood); *C* = Hb of COOLEY's disease; *IE* = Congenital hemolytic spherocytic jaundice.

Finally the paper strips were dried at 80°C and the spots evidenced by naphtalene black. The Hb solutions were obtained from patients suffering from COOLEY's anemia, and from the umbilical cord and prepared by a method used also by SPAET[3].

The results obtained may be summarized as follows:

(1) In agreement with previous observations of REYNAUD, the migration speed of adult Hb is greater than that of the fetal, whereas the migration of COOLEY's anemia Hb has an intermediate rate.

(2) We have also noticed that the pattern of the spots, after evidencing with naphtalene black, was characteristically different for each kind of Hb considered (Figure).

(a) Adult Hb, besides its farther migration from the starting line, appears like a single and well defined band. (Identical behaviour has shown the Hb in one case of congenital hemolytic spherocytic jaundice.)

(b) Fetal Hb, besides its dislocation nearer to the starting line, shows a darker front band followed by a lighter one with distinct contours: the space between the second spot and starting line is colourless.

(c) The Hb of COOLEY's anemia, besides its intermediated speed between the fetal and adult Hb, shows a front band followed by a lighter one, wich unlike fetal Hb, does not show a sharp contour shading to the starting line.

These findings have been reproduced constantly. The pattern of adult Hb therefore substantiates ITANO's statement that the normal adult Hb is a form that occurs free of other components.

COOLEY's anemia and fetal Hbs, on the other hand, are resolved into two bands: the faster possibly corresponding to the adult Hb, the slower corresponding, in one case, to the fetal, and in the other, to COOLEY's Hb. These two bands, as shown above, have different patterns.

These results, which apparently have been obtained also in the DERRIEN's Laboratory[1], will be further discussed on another occasion.

The electrophoretic behaviour, the different resistance of Hb O_2 to denaturation by acids, and the different resistence of Hb CO to denaturation by alkali and acids, strongly suggest that the COOLEY's and fetal Hbs are different.

L. PEROSA and L. BINI

Institute of Clinical Medicine, University of Bari, March 18, 1954.

Riassunto

Gli autori, basandosi sul fatto che l'Hb alcaliresistente del m. di COOLEY si comporta diversamente dall'Hb alcali-resistente del feto, sia per quanto riguarda la denaturazione dell'Hb O_2 con gli acidi, sia per quanto riguarda la denaturazione dell'Hb CO con gli acidi e con gli alcali, e sia, infine, per quanto riguarda il loro comportamento elettroforetico (su carta), prospettano la possibilità che le due Hb alcali-resistenti siano diverse una dall'altra.

[1] Y. DERRIEN, Personal communication.

In vitro Inhibition of Catalase by Ovomucoid

HARGREAVES and DEUTSCH[1] have discovered that the kochsaft of tumors is endowed with anticatalase activity.

We have found that hen's ovomucoid acquires, after boiling, a strong inhibitory power against catalase. The ovomucoid was prepared as follows: the egg white diluted 1:1 with saline was placed in a boiling water-bath for 15 min in tightly stoppered glass tubes. After filtration through paper, the clear filtrate containing the ovomucoid was used for the assays. In our experiments, 1 ml of filtrate was incubated for 1 h at 0°C with 0·5 ml of 0·02 per cent horse liver catalase (prepared according to BONNICHSEN[2]) and the catalase activity was then determined spectrophotometrically[3].

The anticatalase activity of ovomucoid has been found to be proportional to the time of boiling (Fig. 1 *a*),

[1] J. REYNAUD, C. r. Soc. Biol. *147*, 838 (1953).

[2] L. PEROSA and G. RACCUGLIA, Boll. Soc. ital. Biol. sper. *27*, 1590 (1951).

[3] T. H. SPAET, J. Lab. Clin. Med. *41*, 161 (1953).

[1] A. B. HARGREAVES and H. F. DEUTSCH, Cancer Res. *12*, 720 (1952).

[2] R. K. BONNICHSEN, Acta Chem. Scand. *2*, 561 (1948).

[3] F. ABRIGNANI and V. MUTOLO, Boll. Soc. ital. Patol. *3*, 96 (1953).

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.

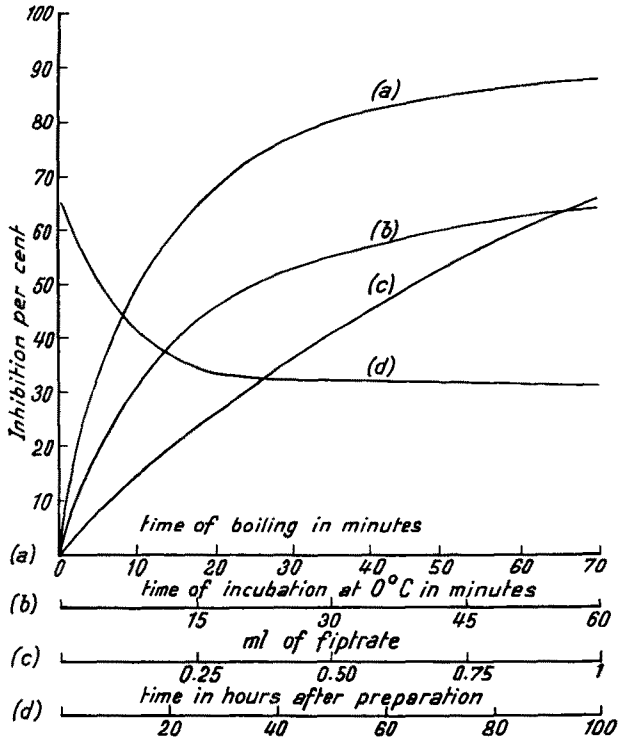


Fig. 1.–Anticatalase activity of ovomucoid. Effect of the time of boiling *a*, of the time of incubation *b*, of amount of filtrate *c* on inhibitory power; spontaneous decline of the inhibitory power *d*.

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.

F. Abrignani and V. Mutolo

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].

[1] V. Erspamer, Rend. sci. Farmitalia *1*, 1 (1954); Ciba Found. Symposium on Hypertension, Churchill, London 1954, p. 78; Pharmacol. Rev. (in Press).

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

[3] V. Erspamer, forthcoming publication.