Wird das Oxim des 4-Pyridyl-glyoxylsäureesters mit verdünnter Mineralsäure in Gegenwart von Thiosemicarbazid gespalten, so decarboxyliert die Säure teilweise, und es entsteht neben dem Pyridyl-mercapto-triazinon auch Isonicotinaldehyd-thiosemicarbazon, ein schon bekanntes Tuberkulostatikum, das sich als Hauptträger der Wirksamkeit erwies[1]. Unter gleichen Versuchsbedingungen wird ein ungefähr konstantes Gemisch der beiden Substanzen erhalten, aus dem sich mit schwachen Basen das Mercapto-triazinon infolge der Schwerlöslichkeit seiner Salze nur schwierig abtrennen lässt. Das reine Pyridyl-mercapto-triazinon schmilzt, aus wässrigem Pyridin umkristallisiert, bei 308° unter Zersetzung (Koflerblock).

Bei keinem der anderen von uns beschriebenen Mercapto-triazinone konnte eine derartige Decarboxylierung beobachtet werden.

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Wissenschaftliche Laboratorien der J. R. Geigy AG., Basel, den 31. Mai 1955.

Summary

In synthesizing 6-(4'-pyridyl)-3-mercapto-1, 2, 4-triazin-5-one from the oxime of 4-pyridyl-glyoxylic acid, isonicotinaldehyde-thiosemicarbazone is obtained through decarboxilation as a by-product, which is mainly responsible for the tuberculostatic properties.

[1] Vgl. J. HIRSCH, Naturwissenschaften *41*, 142 (1954).

Colorimetric and Spectrophotometric Titration of 5-Hydroxyindoleacetic Acid

5-Hydroxyindoleacetic acid (5-HIAA) has been shown to be a normal constituent of the urine of omnivorous and carnivorous mammals and probably of other Vertebrates[1].

Although it cannot be excluded that 5-HIAA comes from 5-hydroxytryptophan without passing through 5-hydroxytryptamine(5-HT)[2], it seems probable that the main part of the urinary 5-HIAA derives from the oxidative deamination of 5-HT.

The quantitative estimation of the urinary output of 5-HIAA has been suggested as the method of preference for a comprehensive evaluation of the general metabolism of 5-HT in the organism. Of course, we cannot expect to get by this method any reliable information on the turnover of 5-HT in the central nervous system, since brain 5-HT makes up hardly 1% of the total 5-HT in the mammalian organism[3].

The two following procedures may be employed in the quantitative titration of 5-HIAA in pure solutions or in watery eluates of 5-HIAA spots from paper chromatograms.

[1] V. ERSPAMER, J. Physiol. *127*, 118 (1955); Pharmacol. Rev. *6*, 425 (1954). – E. TITUS and S. UDENFRIEND, Federation Proc. *13*, 411 (1954).

[2] S. UDENFRIEND, Personal communication to V. ERSPAMER (1955).

[3] V. ERSPAMER, Medicina, Parma (in the Press). – P. CORREALE, forthcoming publication.

p-Dimethylaminobenzaldehyde reaction. To 2 volumes of the liquid under investigation 1 volume of a freshly prepared 2% solution of *p*-dimethylaminobenzaldehyde in concentrated HCl is added. The tubes are then placed in a water bath at 56–58° for 10–12 h.

The coloured solutions are read, within 3 h, in a Beckman spectrophotometer at 600 mμ. LAMBERT and BEER's law is valid between 2 and 25 μg 5-HIAA per milliliter liquid (Fig. 1).

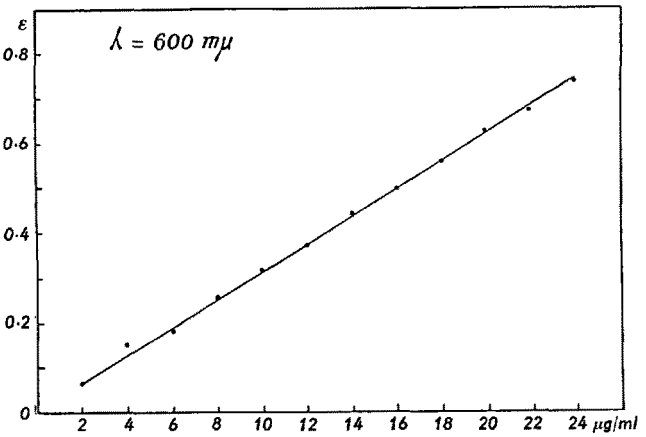

Fig. 1.

Ultraviolet absorption spectrum. Aqueous solutions of 5-HIAA, adjusted to pH 7, show in ultraviolet a maximum of absorption at 221 mμ. LAMBERT and BEER's law is valid between 1 and 15 μg 5-HIAA per milliliter (Fig. 2).

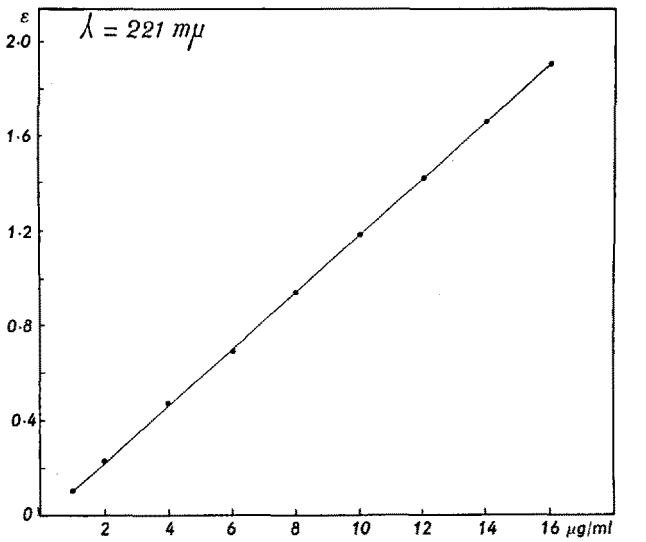

Fig. 2.

Neither of the above methods can be applied directly to biological liquids or crude extracts of biological materials, owing to the presence of disturbing substances. On eluates of urine chromatograms we have succeeded so far in obtaining satisfactory results only with the first method, and only in the case of intense and pure 5-HIAA spots.

P. CORREALE

Pharmacological Institute, University of Bari, Italy, April 25, 1955.

Riassunto

Vengono descritti due metodi, colorimetrico l'uno e spettrofotometrico l'altro, atti alla titolazione quantitativa dell'acido 5-idrossiindolilacetico in soluzioni pure o in eluati, da cromatogrammi su carta, di macchie costituite da acido 5-idrossiindolilacetico.

On the Formation of Coprosterol in the Intestine

Considerable confusion exists in the literature concerning the formation of coprosterol from cholesterol in the intestine.

This is primarily due to three causes:

(1) Failure of attempts to reproduce the process *in vitro* by bacteria (DAM[1]).

(2) The belief that cerebrosides are necessary for the process to occur (ROSENHEIM and WEBSTER[2]).

(3) The belief that cholestenone plays a decisive role as an intermediate (cf. ROSENHEIM and WEBSTER[2]).

The problem has, however, been largely clarified.

In our laboratory it has recently been shown that certain anaerobic bacteria from human feces are able to hydrogenate cholesterol in the form of a colloidal brain suspension to a very high degree (80 to 90%), *in vitro*, whereas several other such bacteria were unable to carry out the hydrogenation under the conditions of our experiments. No hydrogenation occurred with several strains of *Escherichia coli*, several clostridia and bacteroides. A germfree filtrate from human feces was also inactive. The details of this work will be published elsewhere.

Even though our substrate–a brain suspension–contained cerebrosides we do not believe that these substances are essential for the observed hydrogenation of cholesterol.

One of us (DAM[3]) has already shown that, in man, ingested cholesterol was hydrogenated to an extent of more than 80% with diets free from cerebrosides, and ROSENFELD *et al.*[4] have carried out, with feces, the hydrogenation of a colloidal suspension of cholesterol not containing cerebrosides.

The last mentioned authors have shown, by isotope studies, that cholestenone is not a necessary intermediate in the hydrogenation process. It must, therefore, be concluded that the intestinal formation of coprosterol from cholesterol occurs by direct bacterial hydrogenation.

The identification of single strains of bacteria, capable of carrying out the process, is now under investigation.

AGNETE SNOG-KJAER,
INGE PRANGE, and H. DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, May 17, 1955.

Zusammenfassung

Es wurde die Umwandlung von Cholesterin zu Koprosterin *in vitro* durch gewisse anaerobe Bakterien aus Kot durchgeführt.

Stämme von *Escherichia coli*, *Clostridia* und *Bacteroides* erwiesen sich als unwirksam. Die Versuche über die Identifikation der wirksamen Bakterien werden fortgesetzt.

Dosage de la procaïnestérase sérique par spectrophotométrie différentielle

R. HAZARD a montré dans une série de travaux l'intérêt que présente le dosage de la procaïnestérase sérique[1]. La méthode de dosage proposée jadis repose sur la diazotation de l'acide paraminobenzoïque (apab.) libéré au cours de la réaction. Cette méthode, quoique facile à exécuter, est peu apte à des études de nature cinétique. De telles études furent néanmoins commencées[2] et la figure 1 montre la marche de l'hydrolyse d'après les données de HAZARD et RAVASSE (100 γ de procaïne pour 1 ml de sérum).

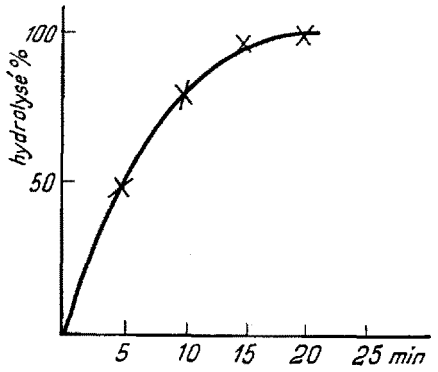

Fig. 1.

Les autres méthodes habituelles de dosage des estérases, comme la méthode manométrique d'AMMON[3] et la méthode titrimétrique ne sont pas applicables[4] à la procaïnestérase, car le pH du milieu augmente au début de l'hydrolyse. Notamment l'acide pab. est un acide faible, tandis que le diéthylaminoéthanol est une base relativement forte. La figure 2 donne les résultats d'une expérience, où le pH du milieu fut enregistré pendant 24 h à 37° (avec un pH-mètre «Radiometer», type 22, muni d'un enregistreur, électrode de verre-pont, agar-électrode de calomel saturée). Le mélange de réaction comportait 1 ml du sérum humain normal, 1 ml d'une

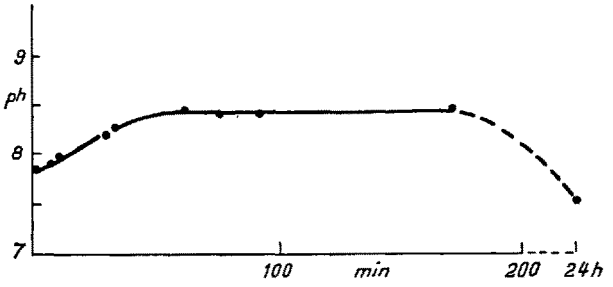

Fig. 2.

[1] H. DAM, Biochem. J. *28*, 820 (1934).
[2] O. ROSENHEIM and T. A. WEBSTER, Biochem. J. *35*, 920 (1941).
[3] H. DAM, Biochem. J. *28*, 815 (1934).
[4] R. S. ROSENFELD, D. K. FUKUSHIMA, L. HELLMAN, and T. F. GALLAGHER, Federation Proc. *13*, 284 (1954). – R. S. ROSENFELD, D. K. FUKUSHIMA, L. HELLMAN, and T. F. GALLAGHER, J. Biol. Chem. *211*, 301 (1954).

[1] R. HAZARD, Presse méd. *56*, 529 (1948). – R. HAZARD, P. NICAUD et A. LAFITTE, Presse méd. *57*, 113 (1949). – R. HAZARD, C. BONAMY et A. CORNEC, Presse méd. *57*, 1133 (1949).
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[3] R. AMMON, Pflügers Arch. ges. Physiol. *233*, 486 (1933).
[4] L. ROBERT, B. ROBERT et M. ROBERT, Bull. Soc. Chim. Biol. *33*, 1859 (1951).