received 400 μmoles/kg Natulan ® or methylamine or 140 μmoles/kg methylhydrazine in one single intraperitoneal injection. For the identification of metabolites in urine, the animals were kept in metabolic cages and the first 24 h urine portion was collected in flasks cooled to 0 °C containing 2 ml of NHCl. The urine was mixed with 9 volumes of chilled ethanol and kept for 15 min at 0 °C and centrifuged. The supernatant was evaporated to dryness at 40 °C under vacuum. Amines and hydrazines present as hydrochlorides in the residue were converted and identified as dinitrophenyl (DNP) derivatives. Urine residues or the synthetic hydrazines or amines were reacted with an excess dinitrofluorobenzene (DNFB) under identical conditions. Because of the relative instability of Natulan ® in solution at alkaline pH[9], the reaction was first conducted for 2 h in a buffer of pH 6.0 (20 volumes 0.1 mol sodium pyrophosphate + 9 volumes 0.3 mol *o*-phosphoric acid). To ensure complete reaction with amines the pH was then raised to 8.5 by addition of a saturated solution of sodium carbonate in 50% ethanol. The reaction was allowed to proceed for 1 h more and the excess DNFB was removed with glycine according to LOCKART[10]. The identity of DNP derivatives obtained from urine was ascertained by thin layer radiochromatography in 5 chromatographic systems using the synthetic ^{14}C-labelled DNP derivatives as reference compounds.

For experiments on the rate and route of excretion of the drugs, animals were put in closed glass metabolic cages provided with an inlet and an outlet for the air. The expired air was aspirated with a water pump, a pressure difference of 45 mm Hg between the air inlet and outlet being used, first through NHCl to absorb bases, then through a trap at − 80 °C, finally through two wash bottles containing 25 ml each of 12% ethanolamine in methanol for the absorption of CO_2. CO_2 was collected continuously up to 14 h after administration, then intermittently. Values obtained after 14 h were interpolated. The total amount of ^{14}C-formaldehyde was determined both in the HCl absorption solution and in the water trapped at − 80 °C. After addition of weighted amounts of carrier formaldehyde, its dimedone derivative was precipitated and recrystallized to constant activity in 50% ethanol.

The radioactivity of CO_2 absorbed in 12% ethanolamine in methanol was measured after addition of one volume scintillation solution consisting of 4 g PPO (2,5-diphenyloxazol) and 100 mg POPOP (1,4-bis-2-(-5-phenyloxazol)benzene) in 1 l toluene. The radioactivity of the other samples was determined after combustion according to the technique of KALBERER and RUTSCHMANN[11]. However, the presence of volatile metabolites made it necessary to omit the drying of samples prior to burning. Radioactivity was measured with a Tricarb-liquid scintillation spectrophotometer (Packard, Mod. 3000).

Zusammenfassung. Nach intraperitonealer Verabreichung von Natulan ®, Methylamin oder Methylhydrazin an Ratten konnte im Urin neben den verabreichten Stoffen Methylamin nachgewiesen werden, dagegen wurde Methylhydrazin als Metabolit von Natulan ® nicht gefunden. Das Auftreten von Methylamin nach Gabe der erwähnten Hydrazinverbindungen zeigt, dass der Rattenorganismus nicht nur, wie bisher bekannt, aromatische Azoverbindungen an der N–N-Bindung spalten kann, sondern auch aliphatisch-araliphatische.

D. E. SCHWARTZ

Research Department of F. Hoffmann-La Roche & Co. Ltd., Basel (Switzerland), January 7, 1966.

[9] K. BERNEIS, M. KOFLER, W. BOLLAG, P. ZELLER, A. KAISER, and A. LANGEMANN, Helv. chim. acta *46*, 2157 (1963).
[10] I. M. LOCKART, Nature *177*, 394 (1956).
[11] F. KALBERER and J. RUTSCHMANN, Helv. chim. acta *40*, 1956 (1961).

Combined Autoradiography and Fluorescence Microscopy. Localization of Labelled 5-Hydroxytryptophan in Relation to Endogenous 5-Hydroxytryptamine in the Gastrointestinal Tract

In a previous paper[1] concerning the distribution of radioactive 5-hydroxytryptophan (5-HTP) and 5-hydroxytryptamine (5-HT) in mice, it was found of value to be able to relate the uptake of the labelled substance to the endogenous monoamines. The present paper describes a combination of autoradiographic techniques with a histochemical method developed by FALCK and HILLARP[2–5] for localization of catecholamines and indolamines.

Since the enterochromaffin cells of the intestine are known to contain large amounts of 5-HT[6–9], these cells have been chosen for methodologic studies of the combination of autoradiographic and fluorescence techniques.

1 mC ^3H-*dl*-5-hydroxytryptophan (G) with a specific activity of 3.6 C/m*M* (Radiochemical Centre, Amersham, England) was given intravenously to an adult female mouse. The dose given was 3 mg/kg body weight. 4 h after injection specimens from the gastric and intestinal walls were freeze-dried and treated with formaldehyde gas[10]. Paraffin sections were mounted under coverslips in liquid paraffin and examined and photographed in the fluorescence microscope. The coverslips were then removed in xylene and the sections were passed down an alcohol series before application of a stripping film emulsion (Kodak AR 10). After exposure (8–10 weeks) and photographic processing some sections were stained, while others were dehydrated and examined unstained in

[1] M. RITZÉN, L. HAMMARSTRÖM, and S. ULLBERG, Biochem. Pharmac. *14*, 313 (1965).
[2] B. FALCK, N.-Å. HILLARP, G. THIEME, and A. TORP, J. Histochem. Cytochem. *10*, 348 (1962).
[3] B. FALCK, Acta physiol. scand. *56*, Suppl. 197 (1962).
[4] H. CORRODI and N.-Å. HILLARP, Helv. chim. Acta *46*, 2425 (1963).
[5] H. CORRODI and N.-Å. HILLARP, Helv. chim. Acta *47*, 911 (1964).
[6] V. ERSPAMER and B. ASERO, Nature *169*, 800 (1952).
[7] R. BARTER and A. G. E. PEARSE, J. Path. Bact. *69*, 25 (1955).
[8] E. P. BENDITT and R. L. WONG, J. exp. Med. *165*, 509 (1957).
[9] V. ERSPAMER, Fortschr. Arzneimittelforsch. *3*, 151 (1959).
[10] B. FALCK and C. OWMAN, Acta univ. lund. *2*, 7 (1965).

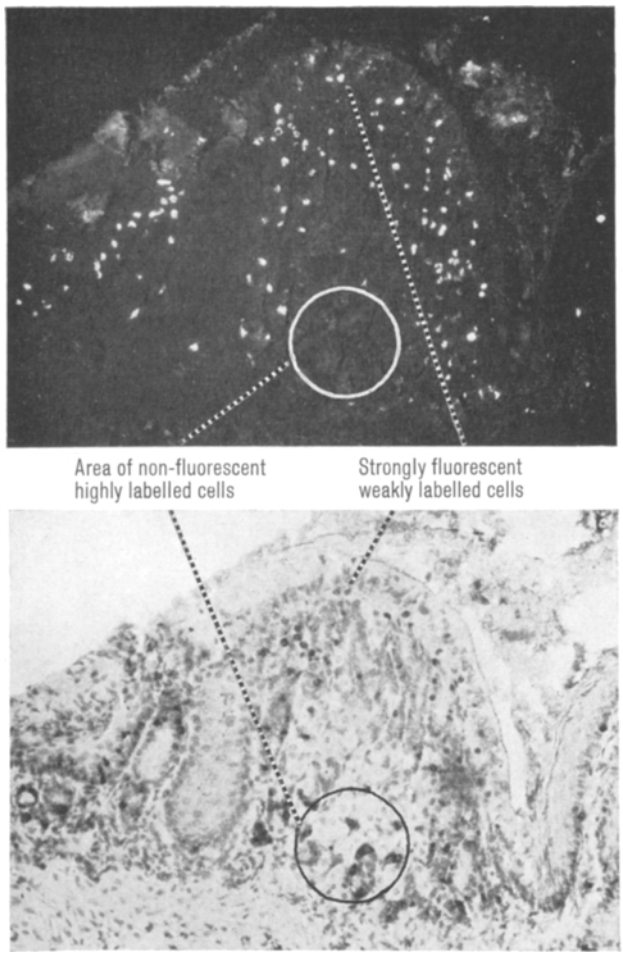


Fig. 1. Section of gastric mucosa from mouse injected with ^{3}H-5-HTP. Fluorescence micrograph (above) and autoradiogram (below). Most of the cells show both fluorescence and uptake of radioactivity but there is generally an inverse relation between the intensity of the fluorescence and the number of silver grains. In the bottom of the crypts a number of highly labelled cells are seen, most of which lack fluorescence. × 150.

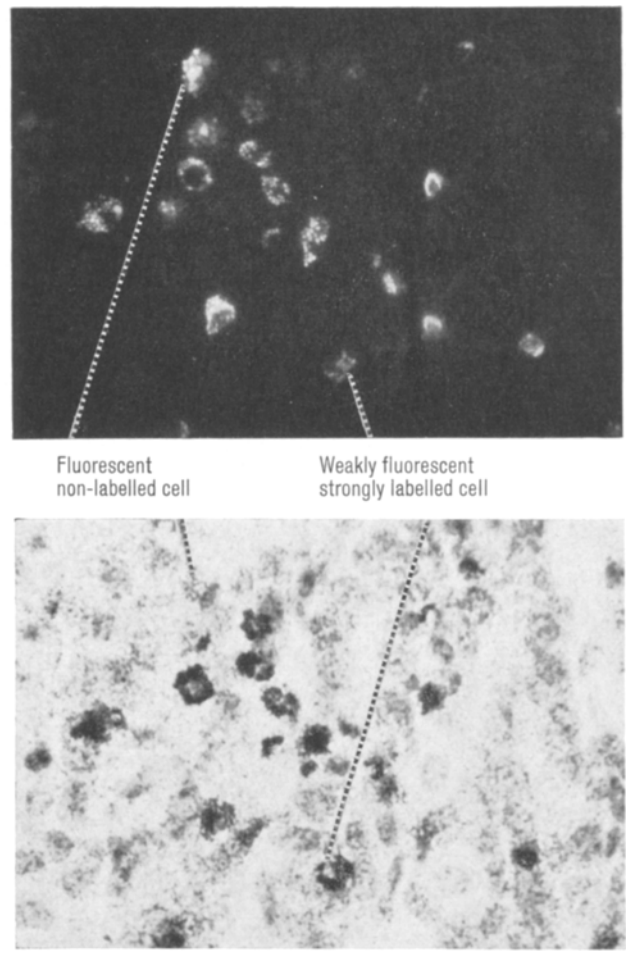


Fig. 2. Detail of Figure 1. Fluorescence micrograph (above) and autoradiogram (below). Most of the cells show both 5-HT fluorescence and radioactivity, but some strongly fluorescent cells have not accumulated radioactivity and some strongly labelled cells show only weak fluorescence. × 430.

the fluorescence microscope for comparison of the fluorescence picture with the autoradiogram.

The autoradiographic blackening and the fluorescence could be seen simultaneously in the UV-light and thus could easily be correlated to each other. However, the quality of the fluorescence picture was better on the photographs of selected areas taken prior to the application of the autoradiographic emulsion. These areas could then be relocated in the autoradiograms.

In the gastric and duodenal mucosa both the fluorescence and the autoradiographic blackening were mainly located to a number of scattered cells probably of enterochromaffin type. The excitation and emission spectra from the fluorescent cells in the gastric mucosa were recorded by means of fluorescence microspectrophotometry[11–13]. They were found to be identical with those obtained from enterochromaffin cells in the duodenum and from 5-HT in model systems. In comparison with duodenal enterochromaffin cells, the gastric cells were more rounded and their granules were considerably larger.

The duodenal cells were more intensely fluorescent than the gastric cells, while the gastric cells showed a much stronger uptake of the labelled substance. Thus there was an inverse relation between intensity of fluorescence and autoradiographic blackening.

The fluorescence seems to indicate the storage of endogenous amine (since the injected small dose of labelled 5-HTP probably did not increase the fluorescence of the cells), and the uptake of radioactivity appears to reflect the rate of synthesis of the amine. Due to the larger cellular depot of 5-HT, more attention has been paid to the duodenal cells when studied by histochemical methods. However, the gastric cells showed a more intense uptake of the labelled precursor and they were larger and more numerous than the duodenal cells. This points to a greater production of 5-HT in the gastric cells.

In gastric mucosa the radioisotope uptake was highest in cells at the bottom of the crypts while fluorescence in-

[11] T. CASPERSSON, G. LOMAKKA, and R. RIGLER JR., Acta histochem. Suppl. 6, in press.

[12] M. RITZÉN, Proc. 2nd Int. Congr. Histochem. Cytochem., Frankfurt 1964.

[13] T. CASPERSSON, N.-Å. HILLARP, and M. RITZÉN, Exp. Cell Res., in press.

tensity was strongest in more superficially localized cells. Some cells showing a strong fluorescence seemed to lack radioactivity and vice versa. The formation of 5-HT thus seemed to be more rapid in young cells located at the bottom of the crypts and then to decrease continuously during the cell migration towards the gastric lumen. The silver grains as well as the fluorescence were located exclusively to the cytoplasm.

HEMPEL[14] recently found a higher uptake of radioactivity in similar gastric cells of mice injected with ^{3}H-dihydroxyphenylalanine (^{3}H-DOPA) than in the duodenal enterochromaffin cells. This indicates that this amine precursor is also taken up more rapidly by the gastric cells.

The rat is known almost to lack enterochromaffin cells in the stomach[15], indicating a species difference. We recently found (unpublished) that ^{3}H-DOPA accumulates in scattered cells of rat gastric mucosa in spite of the hardly noticeable fluorescence. This indicates that there is no species difference as far as cell metabolism is concerned, only with regard to storage capacity of the cells involved.

In the muscular layers of the gastric and duodenal wall a strong accumulation of radioactivity was observed in elongated structures, possibly nerves. A less strong accumulation of radioactivity could be seen in the myenteric plexa. GERSHON et al.[16] have recently reported an uptake of labelled 5-HTP in these ganglia. The localization of a serotonin precursor in the muscular layers of the gastrointestinal tract is of interest when considering the fact that injected serotonin causes contraction of the gastrointestinal wall[17].

Zusammenfassung. Histologische Magen- und Duodenumschnitte der Maus wurden zur gleichzeitigen Lokalisierung biogener Amine und autoradiographischem Nachweis von injiziertem ^{3}H-5-HTP benutzt. Die Radioaktivität war in den enterochromaffinen Zellen lokalisiert und in den Zellen der Magenschleimhaut am höchsten. Fluoreszenz-Intensität und Silberkörnchen-Akkumulation zeigten ein entgegengesetztes Verhalten.

L. HAMMARSTRÖM, M. RITZÉN, and S. ULLBERG

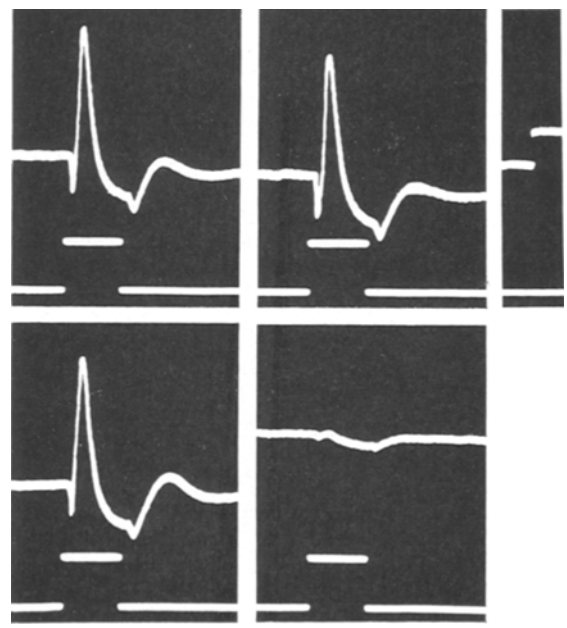
Department of Pharmacology, Royal Veterinary College and Institute for Cell Research, Karolinska Institutet, Stockholm (Sweden), October 6, 1965.

[14] K. HEMPEL, Verh. dt. Ges. Path. *47*, 286 (1963).
[15] W. JACOBSON, J. Path. Bact. *49*, 1 (1939).
[16] M. D. GERSHON, A. B. DRAKONTIDES, and L. L. Ross, Science *149*, 197 (1965).
[17] This work has been supported by grants from the Swedish Medical Research Council Grant No. 12X-713-01 and from Knut och Alice Wallenbergs Stiftelse.

Die Bedeutung von Plasmafaktoren für die isolierte umströmte Kaninchennetzhaut

Die isolierte Warmblüternetzhaut antwortet auf Lichtreize mit einem kompletten Elektroretinogramm (ERG), wenn sie mit einer plasmahaltigen Lösung umströmt wird[1]. Auch die isolierte Kaninchennetzhaut ist mit menschlichem Plasma überlebend zu halten[2]. Auf der Suche nach den wirksamen Faktoren wurden Plasmaderivate verschiedener Species geprüft.

Die früher angegebene Methodik[2,3] wurde modifiziert, indem zur Umströmung eine Mikropumpe (Bühler) benützt wurde, wobei über zwei Doppelhahnsysteme verschiedene Lösungen wahlweise über das Präparat oder über einen Kurzschluss geleitet werden konnten. Als Kontrolle diente das schon in einer früheren Untersuchung[2] verwendete Gemisch von modifizierter Tyrodelösung und antihämophilem Humanplasma (AHP) des Österreichischen Instituts für Hämoderivate; damit wurde bei einwandfrei präparierten Kaninchennetzhäuten bei 30 °C ein ERG erhalten, das aus *a*-, *b*- und *d*-Welle bestand. Die Wirksamkeit anderer Lösungen konnte durch direkten Vergleich mit dieser Standardlösung ge-



ERG der isolierten umströmten Kaninchennetzhaut bei 30 °C. Umströmung mit Tyrodelösung bei Zusatz von 50%: Pferdeplasma (links oben), ultrafiltriertem Pferdeplasma (rechts oben), ultrafiltriertem Pferdeplasma nach 5 min Erhitzen auf 90 °C (links unten), Schweineplasma (rechts unten). Eichung: 0,1 mV. Reizintensität 0,06 lx, Reizdauer 1 sec.

[1] W. SICKEL, H. G. LIPPMANN, W. HASCHKE und CH. BAUMANN, Deutsche Ophthal. Ges. *63*, 316 (1961).
[2] RENATE HANITZSCH und H. BORNSCHEIN, Experientia *21*, 484 (1965).
[3] H. BORNSCHEIN, RENATE HANITZSCH und ASTRID v. LÜTZOW, Experientia *22*, 98 (1966).