percentages of actively phagocyting macrophages were calculated. Similar experiments were carried out with *C. albicans* and nystatin and water-soluble derivative of this antibiotic. After washing the *C. albicans* blastospores cultured previously on Sabouraud's dextrose agar were mixed with mouse peritoneal macrophages and distributed in chambers with cover slips. The slips were removed after 30 min of incubation at 37 °C and washed with warm Parker solution to get rid of non-ingested fungal cells. The slips were placed in fresh culture medium containing appriopriate concentrations of nystatin and at different time intervals they were removed and stained by Giemsa method. The percentages of engulfed *C. albicans* cells actually forming germ-tube were calculated. All tests were performed in triplicate.

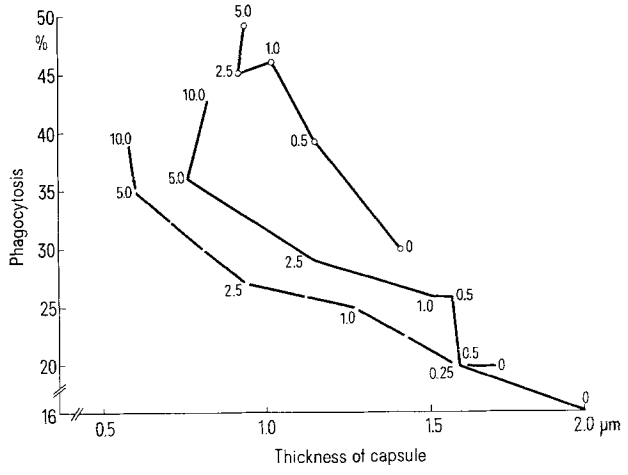

Fig. 2. Correlation between the thickness of capsule in *Cr. neoformans* cells and phagocytosis of these cells by mouse peritoneal macrophages. The numbers at the points of the curve indicate the concentrations (µg/ml) of amphotericin B in the medium in which cryptococcal cells had previously been grown. Each line represents a separate experiment.

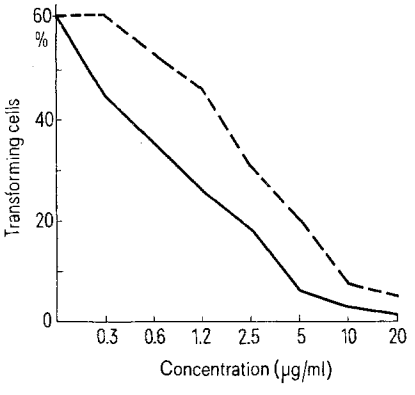

Fig. 3. Influence of various concentrations of nystatin (solid line) and its water-soluble derivative (dashed line) on the formation of pseudomycelia by phagocytized *C. albicans* cells.

Results. The experiments have shown that amphotericin B used in subinhibitory concentrations was able to inhibit the process of capsule formation in cryptococcal cells (Figure 1). It was clearly evident (Figure 2) that such poorly encapsulated cells were more readily phagocytized. Similar results were obtained with polyfungin.

A strict correlation has been observed between the concentration of nystatin in culture fluid and the degree of inhibition of germ-tube formation by engulfed *C. albicans* cells (Figure 3) and as a rule the germ tubes were shorter. This antibiotic showed its action in early as well as in later stages of germ-tube production by *C. albicans* cells while in macrophages. Nystatin in concentration of 10 µg/ml produced complete inhibition of this process independent of whether it was added at the start of the experiment or after 30 and 60 min of incubation.

Discussion. In our previous paper[7] we have shown that nystatin used in comparatively low concentration has the ability to inhibitit the mycelial transformation of *C. albicans* cells suspended in serum. From the experiments presented here, it is clearly seen that nystatin may penetrate to mouse macrophages and inhibit there the process of germ-tube formation by phagocytized *C. albicans* cells. Two other polyene antibiotics inhibited the formation of capsule in *Cr. neoformans* cells thus rendering them more susceptible to phagocytosis. As the ability of *C. albicans* cells to produce germ-tube and ability of *Cr. neoformans* cells to produce the capsule are the determinants of the virulence of these fungi[4,6,8], it is clearly evident that these determinants may be partly or completely abolished by antifungal antibiotics used in comparatively low concentrations. It may be assumed that when thus 'prepared' by an antibiotic fungal cells may be more susceptible to intracellular killing. The results presented might to be an example of a strict collaboration between the mechanism of host immune defence and the activity of antifungal antibiotics.

Zusammenfassung. Nachweis, dass die Kapselbildung von *Cryptococcus neoformans* durch Inkubation mit niedrigen Polyenantibiotika-Konzentrationen gehemmt und die Aufnahme solcher kapselarmer Formen des *Cryptococcus* durch Phagozaten verstärkt wird. Die Hemmung durch das Antibiotikum betraf die Pseudomyzelbildung der intrazellulären *Candida albicans*-Zellen.

J. Borowski, P. Jakoniuk, W. Jabłońska, W. Jakubicz and A. Szpak

Department of Microbiology, University Medical School, Mickiewicza 2, PL-15089 Bialystok 8 (Poland),
25 March 1974.

[7] H. Buluk, A. Boroń-Kaczmarska and J. Borowski, Exp. Med. Microbiol. *23*, 170 (1971).
[8] J. R. Tacker, F. Farhi and G. S. Bulmer, Infect. Immun. *6*, 162 (1972).

Distribution of Radioactivity after Administration of ^{125}I-Labelled Luteinizing Releasing Hormone in Rats

Attention has recently been focused on the entity and duration of permanence of the synthetic gonadotropin releasing hormone (LH-RH) in the pituitary, and also on its possible localization in the cerebral cortex and peripheral organs (e.g., the gonads). Data has already been obtained in similar studies using TRH[1], while, to our knowledge, no study of this type has been carried out using synthetic LH-RH.

The purpose of the present investigation was therefore to study the distribution of radioactivity in various organs following the administration of labelled luteinizing releasing hormone (LH-RH) in rats.

Materials and methods. Adult male and female Sprague-Dawley rats, weighing 250–300 g, were divided into the following lots: 1. normal males, injected with LH-RH-^{125}I; 2. normal females, injected with LH-RH-^{125}I; 3. oestrogen-treated females, injected with LH-RH-^{125}I; 4. controls, males and females, injected with Na^{125}I. In the animals belonging to group 3, 50 µg of oestradiol benzoate and 25 mg of progesterone were injected s.c. 72 h prior to the beginning of the experiment[2].

The LH-RH-^{125}I (s.a. 166–200 mCi/mg) was supplied by Hoechst AG (Frankfurt Main); each rat received 20 ng in 0.5 ml of saline. The control rats were injected with Na^{125}I of the same specific activity (Sorin, Saluggia, Italy).

LH-RH-^{125}I was injected into the carotid artery at time 0, and rats were killed by a guillotine at time intervals of 1, 3, 5, 10 and 30 min; blood samples, hypophysis, muscles, liver, kidney, prostate, cerebral cortex and basal brain including hypothalamus were removed at these times, rinsed with saline, dried, weighed and then put into bacteriological tubes containing 1 ml of Soluene Tm^{100} (Packard), after which they were incubated for 48 h at 56 °C to ensure thorough homogenization. Radioactivity was counted in an automatic gamma-counter (Packard mod. 3002).

The mean of the counts obtained by spectrometry was calculated and the tissue/muscle (T/M) ratio was considered, assuming that muscle is an inert tissue as far as the action of LH-RH is concerned.

Results and comments. The results obtained are listed in Tables I, II and III. Of the organs taken into consideration only the hypophysis, liver and kidney seem to show a selective uptake of the injected radioactivity, and therefore only the figures regarding these organs are reported. The values obtained in other tissues (cerebral cortex, basal brain including hypothalamus, testis, prostate, ovary and uterus) are similar to the uptake values detected in an inert tissue (e.g. muscle).

1. Male rats (Table I). The radioactivity is selectively taken up by the pituitary (T/M 18.20), with a maximum peak 1 min after the injection. It is thereafter rapidly released from the gland, reaching at 3 min 50% of the value at 1 min, and is completely cleared from the blood plasma after 30 min. The kidney also shows a selective uptake of the peptide (T/M 5.88), with a maximum concentration also within the 1st min after the injection and a disappearance curve similar to that of blood plasma. The T/M ratio for the liver is 3.35 and the behaviour of the uptake curve is similar to that of the kidney.

2. Female rats (Table II). 1 min after the injection, the radioactivity seems to be primarily concentrated in the hypohpysis, but the absolute values are higher than those recorded in the male animals (T/M 29.60). The uptake values by kidney (T/M 25.00) and liver (T/M 10.00) are also more pronounced than in the male animals.

3. *Oestrogen-treated female rats* (Table III). The pituitary uptake is markedly higher than in the other groups (T/M 109.00) while the precent uptake by kidney and liver and the drop in the pituitary radioactivity after the 1st min are comparable to those obtained in the other groups.

4. Controls. No significant uptake of radioactivity was observed in the organs of the control animals injected with Na^{125}I.

Our observation of selective concentration of ^{125}I-labelled LH-RH in the hypophysis following s.c. injection is supported by the morphological data of MENDOZA et al.[3], who demonstrated a depletion of peroxidase reactivity in LH-cells of intact animals 1 min after the injection of LH-RH. According to our data, after 1 min the radioactivity leaves the pituitary very rapidly and can be traced in the liver and kidney. Similar results, although with prolonged times (1–3 h), have been obtained by REDDING and SCHALLY[1] with labelled TRH.

Table I. Distribution of radioactivity after injection of ^{125}I-labelled LH-RH in normal male rats

Time after injection (min)	Blood	Liver	Kidney	Hypophysis
1	8.50	3.35	5.88	18.20
3	3.80	2.62	5.20	9.10
5	4.75	2.44	4.70	7.60
10	1.52	1.44	1.00	2.60
30	1.35	0.86	1.29	1.30
	T/M	T/M	T/M	T/M

Values are expressed as tissue/muscle (T/M) ratio.

Table II. Distribution of radioactivity after injection of ^{125}I-labelled LH-RH in normal female rats

Time after injection (min)	Blood	Liver	Kidney	Hypophysis
1	23.50	10.00	25.00	29.60
3	10.50	5.50	11.50	6.30
5	5.90	3.50	5.70	5.50
10	5.00	3.05	4.50	5.50
30	3.60	2.25	2.30	3.00
	T/M	T/M	T/M	T/M

Values are expressed as tissue/muscle (T/M) ratio.

Table III. Distribution of radioactivity after injection of ^{125}I-labelled LH-RH in oestrogen-treated female rats

Time after injection (min)	Blood	Liver	Kidney	Hypophysis
1	39.50	10.40	32.00	109.00
3	8.90	2.40	7.60	8.10
5	8.70	4.30	9.80	7.70
10	5.20	3.10	6.40	3.80
30	4.40	1.50	2.90	2.30
	T/M	T/M	T/M	T/M

Values are expressed as tissue/muscle (T/M) ratio.

[1] T. W. REDDING and A. V. SCHALLY, Endocrinology *89*, 1075 (1971).
[2] S. M. MCCANN and V. D. RAMIREZ, Recent Progr. Horm. Res. *20*, 131 (1964).
[3] D. MENDOZA, A. ARIMURA and A. V. SCHALLY, Endocrinology *92*, 1153 (1973).
[4] Acknowledgment. We are indebted to Dr. F. ENZMANN (Hoechst A.G. Frankfurt Main) for his kind assistance and helpful suggestions.

A selective uptake of LH-RH by the pituitary can therefore be postulated, while kidney and liver may be the primary sites of inactivation and/or removal of the injected material from the circulation.

It remains to be established whether the radioactivity found in the blood, liver and kidney represents all the decapeptide molecule, or if part of it (the active core) remains in the pituitary to exert a long term synthetic action and also a very rapid releasing or activating action.

The concentration of radioactivity in the other organs, including brain and gonads, is negligible. The clinical effects of synthetic LH-RH therefore seem to be mediated through the release of gonadotropins into the blood stream. The administration of the oestrogens seems to 'sensitize' the hypophysis, enhancing its uptake of labelled LH-RH, while kidney and liver are not affected.

Riassunto. Viene descritta la distribuzione della radioattività dopo iniezione intracarotidea di LH-RH marcato con I^{125} in ratti maschi e femmine. L'ipofisi ha mostrato la maggiore capacità di concentrazione dell'ormone marcato, 1 min dopo la somministrazione. L'ormone quindi viene rapidamente dismesso mostrando una riduzione del 50% già dopo 3 min. Altri organi captanti si sono rivelati il rene ed il fegato.

A. Isidori, G. Spera, T. Costa, A. D'Alessio and F. Fraioli

Istituto di Patologia Medica II, Università di Roma, Policlinico Umberto, I–00100 Roma (Italy),
18 May 1974.

Extraction from Hog Duodenum of a High Molecular Weight Protein with Gastric Antisecretory Activity

In 1930 Kosaka and Lim[1] showed that an inhibitor of the gastric secretory activity can be obtained from the intestinal mucosa. Later on (1942–1952), a low molecular weight compound, having peptidic nature according to some authors[2–4] and a non-peptidic one for others[5,6], was believed to be responsible for this inhibiting activity.

Recently, Lucien et al.[7] and Ichimura obtained[8] from hog duodenum an agent very active in blocking the gastric acid secretion. In both cases the inhibitory activity was found to be associated with a low mol wt. compound. In 1970 Brown et al.[9] extracted a polypeptide chain of 43 residues[10] which inhibits gastric secretion.

In this work we show that by using a new procedure it is possible to extract from hog duodenum a protein which behaves as a highly active inhibitor of the gastric secretion and that, in contrast to the above-mentioned agents, it is characterized by a high molecular weight.

Methods. The protein content was determined by the ninhydrin test according to Moore and Stein[11]. The carbohydrate content was determined as follows: hexoses by Winzler[12]; methylpentoses following Dische[13]; hexosamines by the method of Elson and Morgan[14]; sialic acid by Warren[15]; uronic acids according to Bitter and Muir[16]. Sulphate was determined by Terho[17].

Tryptic treatment was performed in 0.24 *M* phosphate buffer, pH 8.3; papain digestion in 0.1 *M* glycine-HCl buffer, pH 2; pronase digestion in 0.1 *M Tris*-HCl buffer, pH 8.7; in all cases at 37 °C for 24 h. The ratio E/S was 1/10.

[1] T. Kosaka and R. K. S. Lim, Proc. Soc. exp. Biol. Med. *27*, 890 (1930).
[2] S. C. Harris, J. S. Gray and A. C. Ivy, Fedn Proc. *1*, 37 (1942).
[3] A. N. Wick, F. Pauls, A. J. Irish and E. M. MacKay, Fedn Proc. *6*, 226 (1947).
[4] H. M. Millen, L. L. Gershbein and A. C. Ivy, Archs Biochem. Biophys. *35*, 360 (1952).
[5] K. J. Öbrink, Acta chem. scand. *2*, 49 (1948).
[6] K. J. Öbrink and H. Winberg, Acta chem. scand. *4*, 789 (1950).
[7] H. W. Lucien, Z. Itoh, D. C. H. Sun, J. Meyer, N. Carlton and A. V. Schally, Archs Biochem. Biophys. *134*, 180 (1969).
[8] M. Ichimura, J. pharm. Soc. Japan *91*, 245 (1971).
[9] J. C. Brown, V. Mutt and R. A. Pederson, J. Physiol., Lond. *209*, 57 (1970).
[10] J. C. Brown and J. R. Dryburgh, Can. J. Biochem. *49*, 867 (1971).
[11] S. Moore and W. H. Stein, J. biol. Chem. *211*, 907 (1954).
[12] R. J. Winzler, in *Methods of Biochemical Analysis* (Ed. D. Glick; Interscience, New York 1955), vol. 2, p. 279.
[13] Z. Dische and L. B. Shettles, J. biol. Chem. *175*, 595 (1948).
[14] L. A. Elson and W. T. J. Morgan, Biochem. J. *27*, 1824 (1933).
[15] L. Warren, J. biol. Chem. *234*, 1971 (1959).
[16] T. Bitter and H. M. Muir, Analyt. Biochem. *4*, 330 (1962).
[17] T. T. Terho, Analyt. Biochem. *41*, 471 (1971).

Table I. Gastric antisecretory activity of fraction E_3 and of fraction E_3 treated with proteolytic enzymes

Treatment	Dose administered as dry weight (μg/kg)	No. of rats	Gastric secretion per 2 h (ml ± S.D.)	Inhibition (%)	Hydrochloric acidity per 2 h (mEq ± S.D.)	Inhibition (%)
Controls		39	3.81 ± 0.43	—	0.201 ± 0.031	—
E_3	500 (385)	37	1.49 ± 0.21	61	0.032 ± 0.005	84
E_3	300 (231)	38	1.79 ± 0.24	53	0.049 ± 0.009	76
E_3	200 (154)	36	1.94 ± 0.30	49	0.057 ± 0.008	72
E_3	150 (115)	37	2.40 ± 0.32	37	0.088 ± 0.014	56
E_3	75 (57)	36	2.74 ± 0.31	28	0.131 ± 0.022	35
E_3, pronase treated	500 (385)	14	3.27 ± 0.40	14	0.165 ± 0.028	18
E_3, papain treated	500 (385)	15	2.97 ± 0.38	22	0.143 ± 0.023	29
E_3, trypsin treated	500 (385)	15	2.68 ± 0.35	27	0.133 ± 0.023	34

In parentheses the protein content.