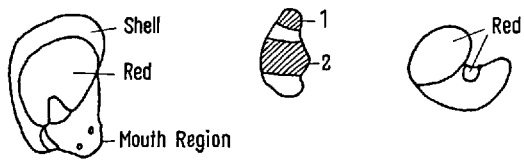
Observations were carried out by putting the embryos with a few drops of water on groove slides. The general contour was clearly visible with a magnification of 60×. After this, more detailed observations could be carried out under a magnification of 240×. The embryos constantly move about giving one the impression that 'they are swimming about within their own eggs'. This makes it difficult to get photographs or even to draw pictures, but it also gives us the opportunity of observing it from different sides and angles.

A series of such observations reveal a remarkable trend of localization of the neutral red in course of morphogenesis. About eight days before hatching, almost the whole embryo is intensely red. At about the time of hatching, in snails which had been stained 144 h earlier, the colour was localized at the site of liver, digestive tube etc. It seems the coloured region lies in the posterior 1/3rd or 1/4th part of the body (see Figure). However, in this region, clear, faint, pink patches of presumably natural colour were also seen when embryos before the hatching stage were stained with Janus green alone. 30 h after hatching, the batch treated with neutral red alone, also showed two blackish band-like regions in the red posterior. Nevertheless, the misinterpretation due to these interferences cannot be very great, because the stain due to neutral red is very bright.



In order to be sure about this progressive localization, near-hatching embryos were kept overnight in neutral red. In spite of this, only the posterior (about 1/3rd part of the body) region was coloured intensely red. Again, a young embryo at the stage of 'eight days before hatching' was kept overnight in neutral red (see Figure). The whole body became red or pink but the most intense parts are shaded. As the region 2 contains the bulk of the body, this may well be intensely red but 1 (the posterior tip) is more intense than the neighbouring region. Two days later, the coloured region was seen to have contracted. It now spanned the region from the posterior up to almost the border of the eye. Near this border region, there were some points of concentration, but they were much less intense than the posterior region. The total coloured region was less than in the near-hatching embryos mentioned above. Four days before hatching, the colour receded to the posterior region only. At this stage, it was again kept in neutral red overnight, till it became uniformly coloured. A few hours later, on the third day before hatching, the colour was seen to have been sharply localized in the posterior half (see Figure). In the next two days there was progressive localization till the snail hatched. (The distortion and rearrangement in the staining pattern due to centrifuging has been studied but the data are as yet too scanty.)

We thus detect a progressive differentiation in the form of a progressive loss of 'neutral-red positive' reaction of regions other than the liver, digestive tube etc. The best-known differentiation of this type is EBERT's[5] finding that in chick embryos the capacity of synthesizing myosin is progressively restricted to the cardiac region alone.

Now, there are indications that neutral red may stain acid and alkaline phosphatase[6], and lysosomes, presumably because they are rich in the above substances. MINGANTI[7] had shown several years ago that phosphatase in *Limnaea* is synthesized only after the trochophore stage. It is possible that later on a progressive restriction sets in.

Further, EVOLA-MALTESE[8] detected a steady rise in and a certain degree of localization of alkaline phosphatase in sea urchin embryos and MINGANTI[7] detected a similar rise in *Limnaea* embryos, suggesting a 'functional differentiation of the digestive tract'. However, our results indicate that in *Limnaea*, after a certain stage, the progressive loss of neutral-red positive reaction is more striking than the 'functional differentiation'.

Our work will continue with Gomori stain and centrifuging experiments, as soon as a new batch of material is available[9].

Résumé. Les embryons de *Limnaea* ont été colorés au neutral rouge suivant la méthode de Janus Verte, signalée par REVERBERI pour les mitochondries. A un jeune stade (Veliger), l'embryon entier est taché par neutral rouge avec une coloration intense du conduit alimentaire; à un stade plus avancé, la tache se localise dans la partie postérieure et, au stade de l'eclosion à peu près, seuls l'estomac et la région hépatique présentent la tache.

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[9] *Note added in proof:* In subsequent observations, in a few cases, some stain was found in the frontal (foot) region. But the progressive loss of stain in the anterior region of the alimentary system was confirmed in *all cases*.

Relation Between Blockade of H^3-Noradrenaline Uptake and Pharmacological Actions Produced by Phenothiazine Derivatives

It has been shown that chlorpromazine decreases uptake of H^3-noradrenaline by the heart, spleen and adrenal glands when given before the labelled compound. Chlorpromazine does not cause release of H^3-noradrenaline bound in these tissues[1,2]. The phenothiazine derivative chlorpromazine has a variety of pharmacological actions, including antihistaminic, adrenolytic and sedative effects. The present study was carried out to see whether any of these actions might be related to the ability of phenothiazines to affect the uptake of H^3-noradrenaline. Four phenothiazine derivatives, each of which lacked certain actions were tested for their ability to block uptake of H^3-noradrenaline into rat hearts.

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Male rats (Sprague–Dawley) weighing 135–155 g were separated at random into groups of 6 or 8 animals. The drugs were administered intramuscularly, 30 min before 10 μc/100 g of *dl*-7-H³-noradrenaline (13 μc/μg) was given intravenously. 2 h later the rats were killed by a blow on the head and the hearts were immediately removed, washed in water, and homogenized in 10 ml of ice-cold perchloric acid (0.4 *N*). After centrifugation, the supernatant solution was assayed for H³-noradrenaline as previously described[3]. The following phenothiazine derivatives (Table) were tested: Promethazine (Phenergan), 10-(2-dimethylaminopropyl)phenothiazine HCl; promazine, 10-(3-dimethylaminopropyl)phenothiazine HCl; selenopromazine, 10-(3-dimethylaminopropyl) phenoselenazine maleate; chlorpromazine, 10-(3-dimethylaminopropyl) 2-chlorophenothiazine HCl. Dosage is expressed in mg of the free base.

Relation between ability to block uptake of H³-noradrenaline (NA) and other pharmacological effects

Drug	H³-NA in heart mμc/g[a]	Relative uptake (%)	Anti-histamine action	Anti-motor action	Anti-adrenaline action
None	575 ± 20	100			
Phenergan	576 ± 44	100	++++	0	+
Promazine	306 ± 26	53	+	+++	++++
Seleno-promazine	211 ± 43	37	++++	+	++++
Chlor-promazine	284 ± 32	49	++	++++	++++

[a] Mean ± S.E.M.

In one series of experiments the dose of chlorpromazine which gave about 50% inhibition of uptake of H³-noradrenaline in rat hearts was found to be 4 mg/kg. This dosage was then used throughout the study. The Table shows that all of the phenothiazine derivatives tested except Phenergan block the uptake of H³-noradrenaline. This effect on injected H³-noradrenaline is compared with other pharmacological actions. The antihistamine, antimotor (sedative) and antiadrenaline actions have been determined for each drug on the basis of work by GLASSMAN, SEIFTER et al.[4–8]. The gradation used in the Table was provided by Dr. J. SEIFTER.

The ability to lower the concentration of injected H³-noradrenaline does not seem to be related to antihistaminic properties since Phenergan, which has a pronounced antihistaminic action but little antimotor or antiadrenaline action, has no effect on the uptake of H³-noradrenaline. Furthermore, promazine has only a slight antihistaminic action, but reduced H³-noradrenaline concentration to about 50% of control value.

The antimotor actions of phenothiazine derivatives also seem to be unrelated to the ability to decrease the tissue concentration of H³-noradrenaline. Selenopromazine shows only a slight antimotor action but affects the concentration of H³-noradrenaline.

However, all of the drugs which have antiadrenaline properties (promazine, selenopromazine, chlorpromazine) had a pronounced effect on storage of H³-adrenaline. Phenergan, on the other hand, has only a slight antiadrenaline action and has no significant effect on H³-noradrenaline storage[9].

Zusammenfassung. Es wurden eine Reihe von Phenothiazin-Derivaten auf ihr Vermögen die H³-Noradrenalinaufnahme in das Rattenherz zu blockieren getestet. Dabei hat sich ergeben, dass dieses Blockierungsvermögen weniger mit den antimotorischen oder Antihistamineigenschaften der Substanzen gekoppelt zu sein scheint, sondern eher mit deren antiadrenergischen Wirkungskomponenten.

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Factor in Human Urine Inhibiting Lipid Metabolism

Adipokinetic activity has been found in the urine of humans[1] or experimental animals[2] during fasting or under other conditions leading to increased mobilization of depot fat. We have been able to detect such activity in extracts from the urine of rabbits during fasting[3] or on exposure to low temperatures[4]. However, attempts to demonstrate this activity in the urine of fasting human volunteers or cachectic patients gave variable results: occasionally the extracts proved adipokinetically active but in other experiments they depressed lipid mobilization. These results suggested a search for an additional factor or factors antagonizing the effect of the urinary adipokinetic factor.

Extracts were prepared from the pooled urine collected from (a) 20–80 healthy human volunteers during the last 16 h of a 36 h fast, (b) 3–5 cachectic patients suffering from malignant neoplasms, over the course of 2–3 weeks, (c) 20 healthy persons on a normal diet (control group). The extracts were obtained by adsorption on benzoic acid and further treatment following the procedure of CHALMERS et al.[1], except that carboxymethylcellulose rather

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