Strahlentherapie bietet er für die Regeneration des Epithels einen wichtigen Hinweis. (f) Nach Abschluss der Strahlentherapie ist das eventuelle Wiederauftreten von atypischen Zellen von grösster Wichtigkeit.

Summary. (1) The vaginal smears show no definite criteria of susceptibility to radiation except for the disappearance of the atypical cells. (2) Nothing can be said about the diagnosis. Only the clinical status may enable the experienced gynaecologist to predict the prognosis. (3) The cytology of the vagina serves as an important guide to the progress of cases of carcinoma which had been radiated or operated. The diagnosis of recurrence of the local, highly differentiated carcinoma, following radiation may present difficulties.

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PRO EXPERIMENTIS

Reliability of Intraperitoneal Injections in Fish

In the study of dose-response phenomena, fishes often display large variances of response in comparison to other test animals. Of course, the sources of error differ as widely as experiments but the technique of i.p. injection is common to the majority. It is possible that a substantial part of the variance in widely different kinds of response emanates from errors of the common technique.

In most cases, it is not possible to use any depot but the i.p. Earlier it has been observed[1,2] that neither of the 2 approaches to the coelomic cavity is very satisfactory: both transparietal and transintestinal injections may be subject to leakage. Here, the leakage of various potentially useful volumes of a transparietally or transintestinally injected solution of radioiodine-tagged thyrotropin has been determined quantitatively. Thyrotropin was chosen because of its intermediate molecular size (mol wt. 28,000). Twenty min after injection, the radioactivity of the aquarium water was determined. It is believed that this time interval constitutes a suitable compromise; the degradation of thyrotropin should be minimal while leakage should be almost terminated.

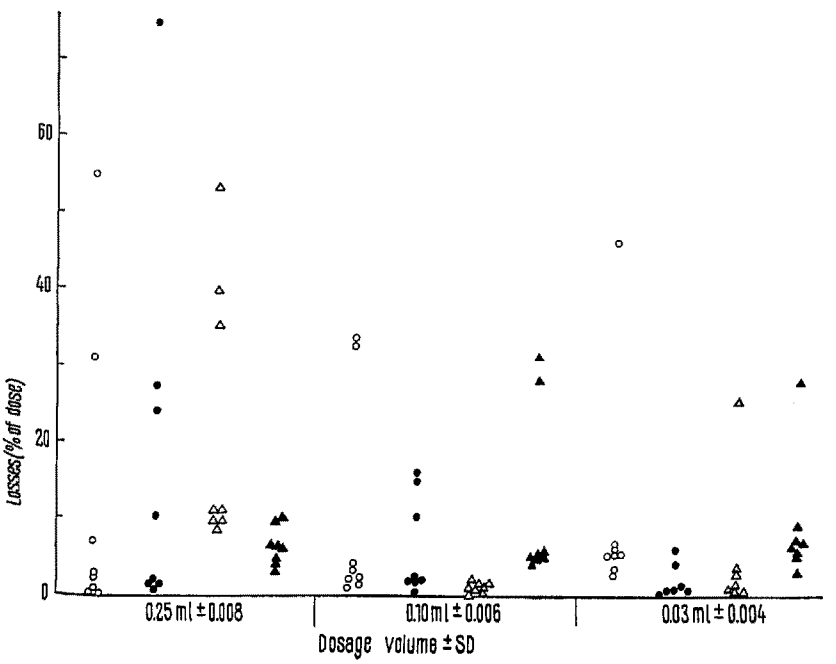
Two means of preventing leakage were devised and evaluated.

Materials and methods. Thytropar (Armour) was tagged with ^{125}I according to OCHI[3]. After careful dialysis it was diluted with 0.70% saline, pH 7.0. Each of the various volumes studied held 150 µg hormone. The injections were given with a 1.0 ml syringe, graduated to 0.01 ml and fitted with a cannula of 0.50 mm O.D.

Carassius carassius L. with a mean weight of 14.1 g ± 2.4 (SD) were used. During experiments, they were kept in individual plastic containers with 300 ml tap water at 21 °C.

In a first screening experiment, 96 fishes were randomly allotted to 12 groups of 8 containers. Four groups were injected with 0.03 ml, another 4 with 0.10 ml and the last 4 with 0.25 ml. Within each set of 4 groups, the injections were given transparietally in 1 pair and transintestinally

[1] W. Chavin, J. exp. Zool. *133*, 259 (1956).
[2] B. M. Dobyns, in *The Pituitary Gland*, (Eds. G. W. Harris and B. T. Donovan; Butterworths, London 1966), vol. I, p. 411.
[3] Y. Ochi, Endocr. jap. *11*, 275 (1964).



Percentage of dose lost during the first 20 min after injection. ○ denotes transintestinal injection, ● dito in silicone-pretreated fish; △ denotes transparietal injection, ▲ dito with film seal.

in the other. Prevention of leakage was tried in 1 group of each pair.

Prevention of transparietal leakage was tried as follows. A small drop of Eastman 910 adhesive was applied to the skin puncture site. A piece of X-ray film (diameter 5 mm) was placed over the glue, which rapidly polymerizes with slight pressure. It will stick in place for several hours. In transintestinal injections, the cannula was introduced into the cloaca and made to pierce the intestinal wall about 5 mm proximal to the vent. Prevention of leakage was tried in the following way. The cloaca was filled with silicone fluid (Midland Silicones, MS 200/100,000) via a blunt cannula prior to the injection of thyrotropin. Due to its high viscosity (100,000 centistokes), the fluid slowly pours out of the cloaca, which is completely emptied 2–3 h later.

Twenty min after injection, duplicate water samples were obtained. The radioactivity of these samples and suitable standards was determined in a well-type scintillation detector.

The 2 most promising techniques were chosen after inspection of data. These experiments were duplicated with 10 fishes/group. In this case, the fishes were killed 20 min after injection by addition of MS-222 (Sandoz) to the water. Five min later, the dead fishes were transferred to Pyrex tubes and dissolved in $2N$ NaOH. The radioactivity of samples of water and dissolved fish were determined as above.

Prior to these experiments, the author had performed several 1000 i.p. injections on small fish.

Results and discussion. The variation between duplicate water samples corresponded to a coefficient of variation $(SD/\bar{X})$ of $< 3\%$.

The results of the screening experiment are illustrated in the Figure. They are very likely representative for most species of fish. Obviously, it is absolutely necessary to pay attention to the reliability of i.p. injections.

The leakage of injected fluid probably depends on raised intracoelomic pressure and delayed closure of cannula canals. Although the pressure increment would be minimal for small injection volumes, the problem of increasing the reliability is not solved simply by using as small volumes as possible: the relative errors of dispensation increase with decreasing volume (Figure, bottom line).

Film glued over the transparietal puncture diminishes the proportion of major leakages but it does not assure total retention of injected fluid. This may be due to leakage prior to application of the seal and raised intracoelomic pressure during postinjection handling. The technique of silicone pretreatment does not suffer from these drawbacks but the silicone plug may have less ability than the film to withstand the rise of pressure induced by large injection volumes (cf. Figure). When large injection volumes are necessary, for instance in the bioassay of hormones in serum, the film technique merits consideration. Incidentally, the film may serve as a means of marking fishes individually.

The transintestinal injection of 0.03 ml in silicone-pretreated fish and the transparietal injection of 0.10 ml were regarded as the most promising techniques. These 2 variants were evaluated with respect to retention in the second experiment. The retentions observed were 94.1% of dose $\pm$ 8.2 and 79.2 $\pm$ 32.4. Thus, the use of small injection volumes, deposited transintestinally with a sufficiently precise syringe in silicone-pretreated fish appears the technique of choice.

By varying the amount and the viscosity of the silicone fluid, its time of stay in the cloaca may be regulated within wide limits. Probably it is unwise to interfer with intestinal flow for more than a few hours. The released silicone floats and may be recovered. Both means of prevention of leakage appeared indifferent.

Résumé. L'hormone thyréotrope ^{125}I fut injectée en doses variées dans le péritoine de Cyprins. Les pertes de substance par écoulement dépassèrent le 50% des doses. Les meilleures rétentions furent obtenues avec de petits volumes, injectés à travers l'intestin, après bouchage du cloaque avec du liquide silicone.

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Continuous Quantitative Recording of Changes in Vascular Permeability

Increased vascular permeability may be induced in the skin of laboratory animals by a variety of agents[1,2], the increased permeability often being demonstrated by the local exudation of a circulating aniline dye such as Evans blue[3,4]. However, the intracutaneous injection of such agents (e.g. histamine) evokes a permeability response whose rapid initial development cannot be demonstrated with sufficient quantitative precision by the dye technique. To overcome this disadvantage, we have therefore developed a method for the quantitative and continuous recording of local changes in vascular permeability.

Circulating bovine serum albumin (BSA), labelled with P^{32}-phosphanilic acid, is used as the 'marker' of increased permeability. P^{32} emits only β-particles of short penetrating power (8 mm in water[5]). It is therefore suitable for the scanning of skin, since radiation from the underlying tissues is absorbed. S^{35} is another pure β-emitter, and S^{35}-sulphanilic acid is also being investigated as an alternative marker.

P^{32}-phosphanilic acid is synthesized by reacting P^{32}-phosphorus trichloride with *p*-nitrobenzene diazonium fluoroborate and hydrolysing the product to P^{32}-*p*-nitrobenzene phosphonic acid, which is subsequently reduced to P^{32}-phosphanilic acid. The diazotized P^{32}-phosphanilic acid is then coupled at pH 9 to BSA and small molecular substances removed by gel filtration.

1 D. L. WILHELM, Pharmac. Rev. *14*, 215 (1962).
2 W. G. SPECTOR and D. A. WILLOUGHBY, Bact. Rev. *27*, 117 (1963).
3 A. A. MILES and E. M. MILES, J. Physiol. *118*, 228 (1952).
4 R. A. RAWSON, Am. J. Physiol. *138*, 708 (1943).
5 H. LEVI, in *Documenta Geigy Scientific Tables* (Ed. K. DIEM; J. R. Geigy S.A., Basle 1959), p. 98.