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.

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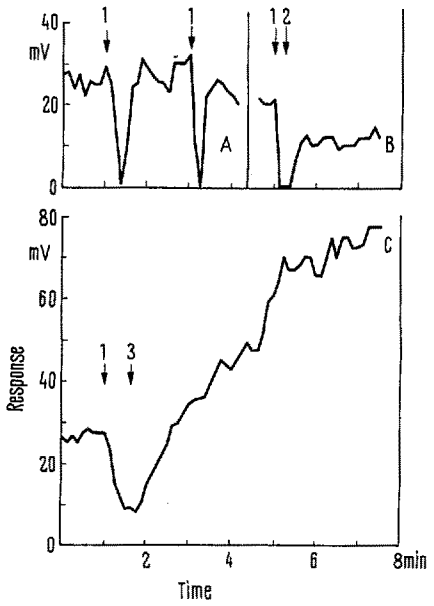
Depilated albino guinea-pigs (500–600 g body weight) are injected i.v. with about 1 ml of the labelled protein solution of approximately 200 μ curie/ml. Thirty min after injection, the animals are tied, back uppermost, to a firm frame. A disc of soft lead (8 cm diameter × 1.5 mm thickness) with a central hole (16 mm diameter) is sandwiched between 2 strips of adhesive tape cut to expose the central hole. The resulting 'belt' is laid on the back of each animal, with the hole exposing a selected area of skin which is then outlined with a fine felt marking pen.



Continuous recordings of changes in vascular permeability. Block (A) illustrates the return of the recording to its previous level following the removal of the test animal (1) and its replacement under the G-M counter. Block (B) records the effect of 0.1 ml physiological saline injected intracutaneously (2), and block C the effect of 0.2 μg histamine (base) in 0.1 ml saline (3). The fall in the recording at (1) in blocks B and C is due to partial removal of the guinea-pig from under the counter to inject saline and histamine respectively. The animal was then immediately re-positioned with the injected site directly under the counter.

A G-M counter with a thin mica end-window (Mullard type MX123) is clamped about 3 mm above the exposed skin and connected to an EKCO type N624 Ratemeter, which in turn is connected to a strip chart recorder; a full deflection of the ratemeter (0–100 pulses/sec) is equivalent to a displacement on the recorder of 110 mV.

When the animals were re-positioned under the G-M counter, the baseline of the record always returned in 45 sec or less to within $\pm$ 3 mV of its previous level (block A in Figure).

When physiological saline alone was injected, the tracing took 30–60 sec to stabilize at a new level, usually several mV below the original baseline (Figure, B). The injection of 0.2 μg histamine (base) induced a rapid rise in the level of the recording (Figure, C), typically 40–45 mV above the baseline. Such increases occurred in 4–5 min, the tracing then remaining at the new level for at least 30 min.

The older methods of assessing increased vascular permeability by the extravasation of serum albumin labelled with an aniline dye or I^{131} do not permit precise observations of the early development of the response. The present method overcomes this disadvantage by permitting the quantitative and continuous monitoring of changes in vascular permeability. By the use of labelled antigen or antibody, this technique may also prove useful for similar studies of immunological reactions in the skin of laboratory animals[6].

Zusammenfassung. Es wird die Anwendung radioaktiven Phosphanilsäure-Rinderserum-Albumins zur kontinuierlichen Messung von Änderungen der Gefässwandpermeabilität beschrieben.

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A Simple Method for the Collection of Leucocytes from Rat Blood

Leucocyte-rich plasma can be obtained from human peripheral blood in a number of different ways[1,2]. In our laboratory, however, attempts to obtain large yields of rat white blood cells by similar methods[3,4] proved inadequate due to poor sedimentation of the erythrocyte mass. The method described in this report has consistently aggregated Lewis rat red cells, thereby yielding plasma that contained large numbers of leucocytes.

Lewis rats were decapitated 5 min after i.v. injection of 10 U of heparin/g body weight: 8–10 ml of whole blood was obtained in this way from rats that weighed 250-300 g. Aggregation of the rat red cells was accomplished by the addition of serum or plasma from normal humans whose red cells were of group B. It was found that the optimum proportions were 2 parts of human plasma or serum to 1 part of heparinized rat blood. The samples, in 12 ml conical centrifuge tubes, were inverted 3 times and placed at a 45° angle for 1 h at room temperature

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