siderable rostro-caudal tongue movement in teleost fishes[5-7].

The explanation of muscle activity and structure of joints will be published later, together with the results of vertical X-ray pictures. Here it suffices to conclude that this technique permits an easy determination of the movements of the parts in intact organisms. For the generally accepted conception of the double acting pump, those observations have some consequences. They strongly suggest that the respiratory movement must be considered to be at least partly a peristaltic movement[8].

Zusammenfassung. Ein einfacher Apparat ermöglicht mit direkter Röntgenbestrahlung kinematographische Aufnahmen von Tierbewegungen. Der Apparat besteht aus einer Drehscheibe und einer Säule von Zahnröntgenfilmen, welche durch die Scheibe jeweils nach Belichtung entfernt wird. Als Beispiel wurden Fisch-Atmungsbewegungen analysiert.

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Zoological Laboratory, State University, Leiden (The Netherlands), August 23, 1966.

5 O. Holmquist, Acta univ. lund. *2*, 6 (1910).
6 V. V. Tschernavin, Proc. zool. Soc. Lond. *118* (1949).
7 W. H. van Dobben, Thesis, Utrecht (1935).
8 We are very thankful to J. Altink, W. Duk, F. J. S. W. Körner and A. L. C. Heemskerk for the construction of the apparatus and their critical and stimulating discussion during its design. We also thank C. Elzinga for preparing the drawings and Drs. C. Gans and H. J. de Jongh for their valuable correction of the English text.

Immunofluorescent Technique to Detect Anti-Penicillin Antibody

A number of tests with immunofluorescent methods have been performed in order to achieve a simple and quick technique for investigating immunological cross reactions between different antibiotic molecules.

We have obtained an easy indirect method, which we hope will resolve this kind of problem, in which the antibiotic molecule is fixed on rabbit erythrocytes to dispose of an antigen clearly visible through the microscope. Specific immunosera are mixed with them on smears and afterwards an anti-rabbit fluorescent-labelled serum is used.

The materials used in the biological test are: (a) *erythrocytes* from rabbits in which penicilolyl-polylysine molecules[1] have been fixed. These were prepared by mixing rabbit blood with an equal volume of modified Alsever's liquid[2]. After washing the erythrocytes several times in buffered saline solution, 1 ml was maintained with 9.0 ml of 10^{-6} *M* peniciloyl-polylysine solution (Cilligen), first at 37 °C for 30 min and then at 4 °C overnight. Later the erythrocytes were washed 6 times in buffered saline solution and were then ready for use.

(b) *Anti-drug immunosera.* These were obtained by inoculating rabbits with penicillin plus Freund complete adjuvant mixture[3] so that the rabbit received 125 mg penicillin plus 1 ml of adjuvant in a volume made up to 2 ml with saline. This volume was injected into the pad of each foot and at 4 points on the rabbit's back. During the first 4 weeks the animal received 2 weekly injections and then 2 more at 10 day intervals. Samples of blood from each rabbit were taken by heart puncture 10 days after the last injection and the antibody titre was found by hemagglutination of these sera against penicillin-coated erythrocytes[4]. When the titre was high enough, the rabbits were bled to death.

(c) *The goat anti-rabbit globulins* (provided by the Pasteur Institute). These were labelled with fluorescein isothiocyanate at a rate of 1/20.

The method we propose is as follows: a smear of peniciloyl-polylysine coated erythrocytes is fixed with acetone at − 20 °C for 10 min and then treated with anti-penicillin rabbit serum for 30 min at 37 °C. After washing in buffered saline solution, it is stained by fluorescent anti-rabbit reagent for 30 min at 37 °C and then washed again and mounted with buffered glycerine, pH 9.0, to be

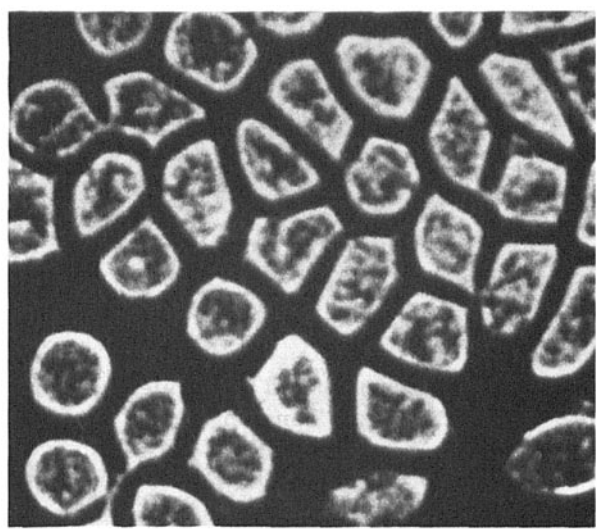

Figure a

1 B. C. Brown, E. V. Price, and M. B. Moore jr., J. Am. med. Ass. *189*, 599 (1964).
2 S. C. Bukantz, C. R. Rein, and J. F. Kent, J. lab. clin. Med. *31*, 394 (1946).
3 P. Bunn, L. Canarile, and J. O'Brien, Proceed. IIIrd Intern. Congr. Chemoth. *2*, 1442 (1963).
4 J. A. Thiel, S. Mitchell, and Ch. W. Parker, J. Allergy *35*, 399 (1964).

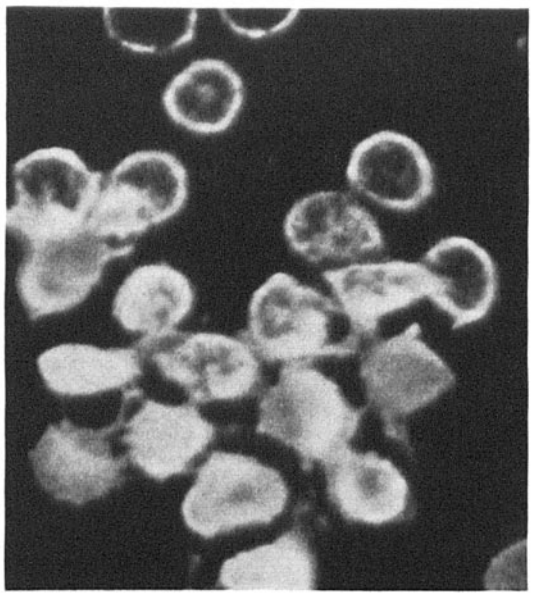

Figure b

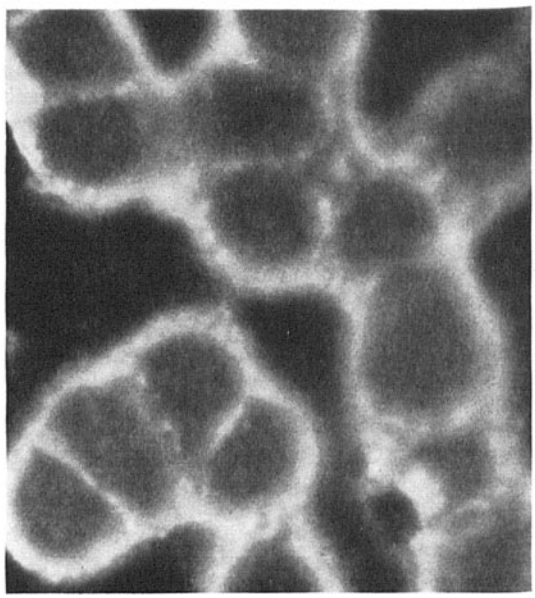

Figure c

observed under the microscope. (We used an Ortholux Leitz microscope with an HBO 200 W lamp and a BG 3 UV-filter.)

The Figures *a* and *b* (× 1800) show positive results with this technique. In Figure *c* (× 3000) can be clearly noted a strong fluorescence just at the points where the penicilyl-polylysine molecules are fixed on the erythrocyte membrane and therefore where the specific reaction takes place.

Since we have failed in our previous assays using penicillin instead of cilligen as antigen, we consider the present technique to be a useful tool for studying antigenicity in complete and modified antibiotic molecules.

Resumen. Se propone un sencillo y rápido método de inmuno fluorescencia para detectar anticuerpos antipenicilina: Frotis de eritrocitos de conejo, sobre los que se han fijado moleculas de peniciloyl-polylisina, son tratados con suero de conejo inmunizado y despues con suero de cabra anticonejo marcado con isothiocianato de fluoresceina. De este modo se observa acusada fluorescencia en los casos positivos.

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Intraluminal Pressure and Equivalent Arterial Wall Tension

Mechanical properties of the arterial wall can be studied conveniently on two types of isolated preparations: isolated segments and helically cut strips of artery. In a segment of artery filled with Ringer's solution the transmural or intraluminal pressure (P) acts radially from the aqueous phase into the tissue phase attempting to increase the radius. P is equilibrated by the circumferential elastic tension (T) developed within the tissue phase itself at right angles to the radius (Figure 1c, top). Physiological workers usually determine P, and then calculate T from a law of Laplace, $P \cdot r = T$, r being the radius of the arterial segment (see BURTON[1]). A formula modified to include the thickness of the arterial wall was used by PATTERSON[2].

It was possible to bring about an experimental situation in which a value of intraluminal pressure, P, and its equivalent tension, T, of the vascular wall can be measured. k, the correlating factor from $P \cdot k = T$, can thus be compared with r, the radius measured at P pressure. In other words, an experimentally determined T value can be compared with a T' value computed by use of Laplace's formula $P \cdot r = T'$.

Methods. The isolated helically cut aortic strip of the rabbit was prepared as described before[3]. A strip 4 mm wide was suspended in Krebs-HCO_3-Ringer solution maintained at 37 °C, and aerated with a gas mixture of oxygen, 95%, and CO_2, 5%. The strip was extended to a tension

[1] A. C. BURTON, in *Handbook of Physiology, Circulation* (American Physiological Society, Washington, D. C. 1962), vol. 1, p. 85.

[2] L. H. PETERSON, R. E. JENSEN, and J. PARNELL, Circulation Res. *8*, 622 (1960).

[3] M. WURZEL, T. P. PRUSS, W. WEISS, and G. D. MAENGWYN-DAVIES, Proc. Soc. exp. Biol. Med. *105*, 659 (1960).