fibrinolytic activity in the valves would not remain after the sterilization procedures used in preparing them as prostheses for human aortic valves[9].

Zusammenfassung. Herzklappen vom Schwein wurden auf ihre thromboplastische und fibrinolytische Aktivität untersucht. Die Konzentration von Gewebsthromboplastin war in allen Herzklappen (Segel- und Taschenklappen) gering und stimmt mit der in menschlichen Herzklappen gemessenen überein. Im Gegensatz dazu weisen Schweineherzklappen eine höhere fibrinolytische Aktivität als menschliche Herzklappen auf, wahrscheinlich als Folge der reicheren Vaskularisierung.

PIA GLAS and T. ASTRUP

The James F. Mitchell Foundation, Institute for Medical Research, Washington (D.C. 20015, USA), 28 October 1968.

[9] Supported by grant No. HE-05020 from the U.S. Public Health Service, National Institutes of Health, National Heart Institute.

The Transport of Various Immune Globulins by the New-Born Pig Intestine

The new-born pig obtains a passive immunity to disease by the ingestion and subsequent intestinal absorption of antibodies present in the sow's colostrum. The appearance of the intestinal mucosa shortly after birth suggests that protein is being endocytosed by the mucosal cell[1], but the mechanism which initiates endocytosis or the means by which protein leaves the cell is not known. Any demonstration of selectivity during absorption would help to define conditions critical for the movement of protein molecules. The present work was therefore designed to test whether immune globulins derived from different species would be transported at different rates by the pig intestine used in vitro.

Materials and methods. Piglets collected at birth before they could suck the sow were killed by decapitation and everted sacs of small intestine formed and incubated in bicarbonate saline as described previously[2]. Incubation was for 2 h at 37 °C in medium containing glucose, 1 mg/ml, together with a known preparation of IgG (final concentration 5 g/100 ml). Fluid removed from the sacs at the end of incubation was weighed and samples later analysed for sodium using an EEL flame photometer, for glucose using the glucose oxidase method of HANSEN[3] and for IgG using the quantitative immunodiffusion assay of GELL[4]. Intestinal sacs were weighed at the end of incubation and serosal transfers calculated as amount transferred g intestine/h incubation. Values have been represented in each case as per cent change from values found in the presence of porcine IgG to lessen errors due to animal variation. Direct comparisons were made between alternate sacs, one incubated in bicarbonate saline containing porcine IgG and the other incubated in saline containing IgG prepared from a different species. The significance of any difference was assessed using the Wilcoxon test[5]. Human, equine and ovine γ-globulins (Koch-Light Labs. Ltd.) were stated to contain $\geq 98\%$ γ-globulins estimated by electrophoresis on cellulose acetate. The purity of bovine and porcine γ-globulins (Armour Pharmaceutical Co. and Pentex Inc. respectively) was stated to be $> 95\%$. All preparations had been manufactured using the same Cohn fractionation technique.

Results and discussion. The purity of these various proteins was first assessed by comparing their sedimentation patterns on ultracentrifugation (Figure 1). Protein sedimenting as the major peak corresponded to 7S globulin (IgG globulin). The small amount of material which sedimented faster was thought to consist of aggregated IgG. The proportion of aggregated to 7S IgG was lowest for ovine and highest for porcine preparations, but the difference between individual samples was small. Equine IgG contained about 5% (w/v) sodium chloride and this was removed by dialysis. The proportion of 7S to aggregated IgG was not changed by this treatment or by subsequent freeze drying (Figure 1 compare E with E(d)). Salt-free equine IgG was used for all further work. The homogeneity of these proteins was next assessed by comparison of their immunoelectrophoretic patterns (Figure 2). Antibodies to porcine, equine and bovine IgG globulins had been raised previously in rabbits. Antisera to human and ovine IgG light chains, prepared in sheep and rabbit respectively, were used to precipitate the whole IgG molecules from these species. Antiserum to bovine IgG produced a precipitin line of partial identity with

[1] A. G. M. MATTISSON and B. W. KARLSSON, Arch. Zool. *18*, 575 (1967).
[2] A. E. PIERCE and M. W. SMITH, J. Physiol. *190*, 19 (1967).
[3] O. HANSEN, Scand. J. clin. Path. *14*, 651 (1962).
[4] P. G. H. GELL, J. clin. Path. *10*, 67 (1957).
[5] W. J. DIXON and F. J. MASSEY, in *Introduction to Statistical Analysis* (McGraw-Hill, New York 1957), p. 488.

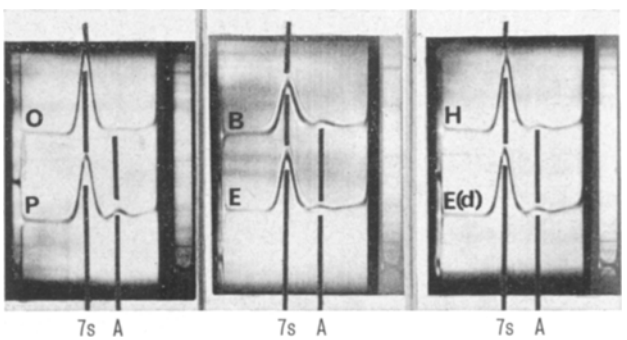

Fig. 1. Ultracentrifugal patterns obtained using commercial preparations of γ-globulins prepared from 5 different species. The final concentration of each protein, dissolved in phosphate buffer $I = 0.1$, pH = 7.5, was 0.8% w/v. Centrifugation was at room temperature for 55 min at 59,780 rpm O, P, B, H and E, ovine porcine, bovine, human and equine γ-globulins. E(d), equine γ-globulin after dialysis. A, aggregated globulin. 7S, globulin having a Svedberg coefficient of approximately 7S.

ovine IgG that ovine IgG contained at least 2 types of IgG molecule. These were tentatively identified as fast IgG (IgG1 nearest the anode) and slow IgG (IgG2), 2 subclasses of IgG normally present in this species. Some of the equine IgG moved faster than that of other species. There were however no gross differences between the 5 protein preparations and experiments were therefore carried out to determine their various rates of transport across the pig intestine.

The mean control transfers measured using bicarbonate saline containing porcine IgG was, for fluid, 0.90 ± 0.03 ml/g · h; for sodium, 145 ± 7.3 μmole/g · h; for glucose, 20.0 ± 1.3 μmole/g · h and for porcine IgG, 82 ± 13.8 μg/g · h (45 determinations ± S.E.). The porcine IgG produced a single precipitin line on immunoassay; there was no evidence to suggest this protein was being degraded during transfer. The serosal transfer of porcine IgG is compared with that for other samples in Figure 3. Bovine IgG was transported more quickly than porcine IgG. The apparent differences between the rates at which ovine, equine and human IgG globulins were transported were not statistically significant. It should be pointed out however that the choice of reference standard, in this case porcine IgG, determines which samples of IgG behave differently. A direct comparison of equine with human IgG globulins for instance, might well have revealed a significant difference between their rates of transport. The serosal transfer of both sodium and fluid was also increased by bovine but not by other preparations of IgG. The serosal transfer of glucose, not shown in Figure 3, remained constant whichever IgG was used in the incubation medium.

A protein-dependent increase in sodium and fluid transfer with glucose transfer remaining unchanged is also found when bovine plasma albumin bathes the everted pig intestine[6]. The intestine of the new-born pig resembles an amoeba in this respect, both albumin and globulin being effective inducers of pinocytosis[7]. But if molecules as different in size as albumin and globulin appear to act in the same way, then why should molecules as similar as bovine and porcine IgG behave differently? One reason might be that the proportion of IgG1 to IgG2 globulin changes from one preparation to another. These 2 globulins are transferred at widely different rates by both bovine[8,9] and ovine[10] mammary epithelia during early lactation and the new-born pig intestine might also select between these 2 sub-classes of IgG. Certainly one should not speak about a species specific transport of IgG until more is known about the rates at which these 2 sub-classes of IgG globulins cross the pig intestine. There is no doubt however that the pig intestinal epithelium can discriminate to some extent between different

[6] P. BROWN, M. W. SMITH and R. WITTY, J. Physiol. *198*, 365 (1968).
[7] H. HOLTER, Int. Rev. Cytol. *8*, 481 (1959).
[8] F. A. MURPHY, O. AALUND, J. W. OSEBOLD and E. J. CARROLL, Archs Biochem. Biophys. *108*, 230 (1964).
[9] A. E. PIERCE and A. FEINSTEIN, Immunology *8*, 106 (1965).
[10] D. D. S. MACKENZIE and A. K. LASCELLES, Aust. J. exp. Biol. med. Sci. *46*, 285 (1968).

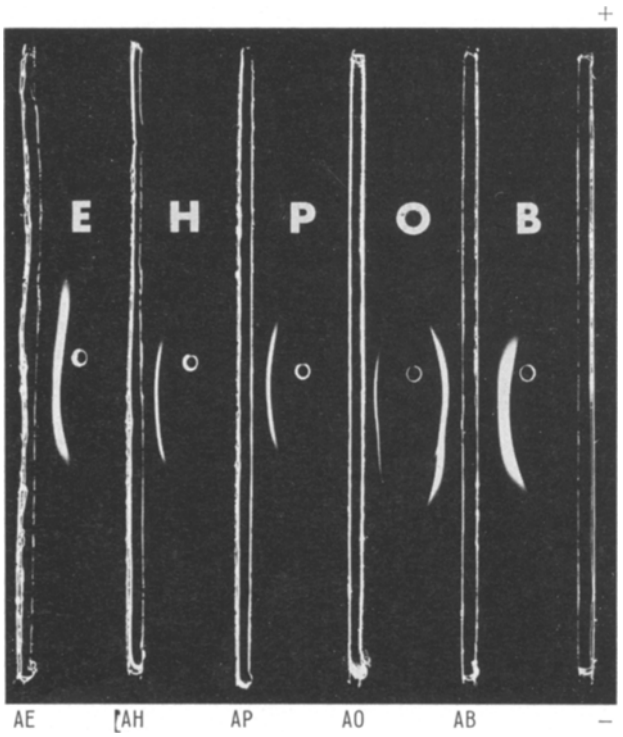

Fig. 2. Immunoelectrophoresis of different IgG preparations. Solutions of equine (E), human (H), porcine (P), ovine (O) and bovine (B) IgG were placed in the wells at a concentration of 1% w/v. Electrophoresis was for 4 h at 7 V/cm in 0.025 *M*-veronal buffer, pH 8.6. Undiluted antisera to the various proteins were then placed in the troughs and 12 h allowed for the precipitin reaction to take place. AE, AH, AP, AO and AB, antisera to equine, human, porcine, ovine and bovine IgG respectively.

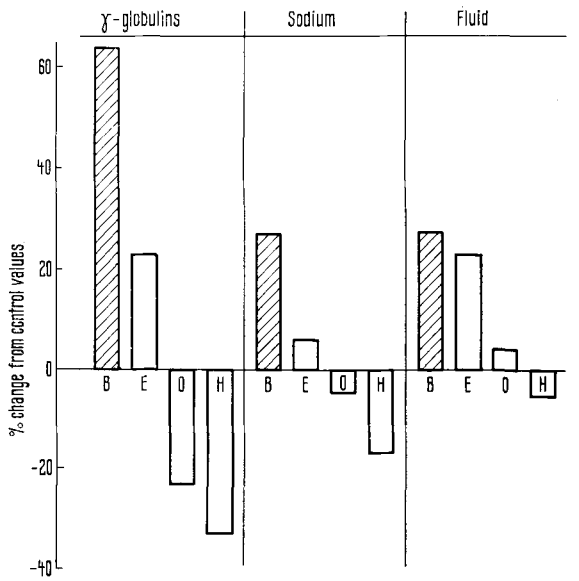

Fig. 3. The serosal transfers of different IgG preparations measured across the everted small intestine of the new-born pig showing the effect on sodium and fluid transport. Everted sacs were incubated for 2 h at 37 °C in bicarbonate saline containing a specified preparation of IgG at a concentration of 5 g/100 ml. Comparisons were made with alternate sacs incubated in medium containing porcine IgG (5 g/100 ml). Each histogram gives the mean percentage change from 11 or 12 comparisons. Open histograms, no significant change; cross-hatched histograms, change significant at the probability level $p < 0.01$ for sodium and fluid, $P = 0.1$ for bovine IgG. B, E, O and H, preparations of bovine, equine, ovine and human IgG.

preparations of IgG globulins and this alone is sufficient reason to question the current belief[11] that the absorption of protein in this species is by a process which is entirely non-selective[12].

Resumen. El intestino delgado del cerdo recién nacido, usado in vitro, transporta immunoglobulina G (IgG) de bovino más rápido que IgG de porcino. El transporte intestinal tanto de sodio como de fluido aumenta en presencia de IgG de bovino. Preparaciones de IgG de ovino, equino y humano son transportadas a igual velocidad que IgG de porcino. Estas proteínas no estimulan la transferencia de sodio o fluido. Esta diferencia en la velocidad de transporte entre IgG de bovino y porcino pone en duda la opinion general que sostiene que la absorción de proteína en el intestino de cerdo es enteramente no selectiva.

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A.R.C. Institute of Animal Physiology, Babraham, Cambridge (England), 14 October 1968.

[11] I. G. Morris, in *Handbook of Physiology-Alimentary Canal* (American Physiological Society, Washington DC 1968), vol. 3, p. 1491.

[12] We would like to thank Dr. A. Feinstein and M. J. Hobart for providing antisera to human and ovine IgG light chains; N. Buttress for his ultracentrifugal analysis of proteins and K. Burton and T. Archer for their technical assistance in this work.

[13] P.I.E.R. Fellow, N.R.C. Canada.

[14] Unilever Fellow.

Transformation of Phage-Resistance in *Bacillus subtilis*

The occurrence of transformation has now been confirmed in different bacterial species. A wide range of bacterial characters are transformable[1].

There are as yet no reports of transformation of phage-resistance, but the present investigations show how transformation of phage-resistance can occur.

For transformation the recipient strain *Bacillus subtilis* 168 M try$^-$ phs (SPO-1 phage sensitive) was used. Phage-resistant spontaneous mutant was isolated from the host bacteria and designated 168 M try$^-$ phr. The mutation rates was 8.32×10^{-6} calculated to Luria and Delbrück[2]. Three bacterial strains were used as donors of transforming DNA. One of them was *B. subtilis* Marburg. The other 2 prototrophic bacterial strains 168 M try$^+$ phs and 168 M try$^+$ phr were obtained by transformation of 168 M try$^-$ phs and 168 M try$^-$ phr spontaneous mutant using DNA of *B. subtilis* Marburg.

The preparation of optimal competent cells and the transformation procedure were previously described in detail[3].

To show that the phage-resistance transformation is connected with the DNA isolated from phage-resistant strain, the following experiment was made. Competent 168 M try$^-$ phs cells were used in the transformation experiment. Three kinds of DNAs were used with a final concentration of about 2 µg/ml. The bacterial-DNA mixtures were shaken for 30 min in the water-bath at 37 °C; and 0.2 ml were then measured into 3 100 ml Erlenmeyer flasks fitted with side arms in which were 10 ml MG liquid media[3] containing 0.1% casein hydrolysate. The incubation was continued till the suspensions reached 0.3 optical density values (9–10 h). The try$^+$ phs transformants were selected on MG agar[3]. The phage-resistant transformants were selected as try$^+$ phr cells using SPO-1 phage as a selective agent permitting the detection of the phage-resistant transformants present in the population. The results are seen in the Table.

The number of try$^+$ phs and try$^+$ phr transformants/ml and the frequency of try$^+$ phr cells after transformation, using different kinds of DNA

	DNA from 168 M try$^+$ phr	168 M try$^+$ phs	*B. subtilis* Marburg
try$^+$ phs	1.23×10^8	8.03×10^7	1.03×10^8
try$^+$ phr	9.20×10^5	2.38×10^3	1.27×10^3
Frequency of try$^+$ phr	7.50×10^{-3}	2.96×10^{-5}	1.23×10^{-5}

It is seen that the frequency of the phage-resistant cells is 7.5×10^{-3} in the case of the bacterial-DNA mixture containing DNA isolated from the phage-resistant strain. In the other 2 cases, where the donor strains were sensitive, the frequencies of the phage-resistant cells are very low. The number of phage-resistant cells in these cases was nearly the same as the number of spontaneous phage-resistant mutants among the 168 M try$^-$ phs cells.

The curve of competence was determined by the number of try$^+$ phs and try$^+$ phr transformants. In both cases the beginnings and the peaks of competence occur nearly at the same time, but do not run parallel with each other.

Transformation procedure was carried out using different concentrations of DNA prepared from 168 M try$^+$ phr bacterial strain. The number of try$^+$ phs transformants gave a straight line in the coordinate, but the curve of the number of try$^+$ phr transformants did not run parallel with it. It seems that the try$^+$ and phr markers are not linked.

The number of try$^+$ phs transformants rose quickly to a high level during transformation and the phenotypic lag was about 5 h. The try$^+$ phr transformants appeared only after 6 h 30 min incubation.

The frequency of transformation for try$^+$ phr marker compared to try$^+$ phs marker was about 0.12–0.032%[4].

Zusammenfassung. Mit Hilfe von extrahierter DNS wurde der genetische «Marker» der Phagenresistenz auf phagensensible Bakterien übertragen.

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Institute of Genetics, Hungarian Academy of Sciences, Budapest (Hungary), 14 October 1968.

[1] A. W. Ravin, Adv. Genet. *10*, 61 (1961).

[2] S. E. Luria and M. Delbrück, Genetics *28*, 491 (1943).

[3] S. Horváth, J. gen. Microbiol. *48*, 215 (1967).

[4] I wish to thank Dir. Dr. B. Györffy for his criticism and Irene Kállay for her assistance.