

Table 4 Parameter Values and Standard Errors for Both Models over 100 Computed Growths up to 1,600 Abnormal Cells

Value of κ	D-M model (4)				Standard error s	W-B model (3)		Standard error s
	A	Parameter values B	C	Parameter values β		α		
∞ (250 growths)	0.23790 (0.23914)	0.51964 (0.50693)	-0.41495	0.00255	0.45323	0.39039 (0.3903)	0.03088	
50	0.24052 (0.24136)	0.53494 (0.52248)	-0.43151	0.00547	0.45303	0.39621 (0.3956)	0.03325	
20	0.24241 (0.24486)	0.56032 (0.54703)	-0.44599	0.00885	0.45091	0.40801 (0.4040)	0.03591	
10 (98 growths)	0.25097 (0.25121)	0.59442 (0.59158)	-0.47121	0.00733	0.4497	0.42778 (0.4192)	0.03561	
5 (96 growths)	0.26926 (0.2663)	0.65041 (0.69740)	-0.51644	0.00526	0.44924	0.46198 (0.4554)	0.04116	
3 (82 growths)	0.29815 (0.29346)	0.87830 (0.88786)	-0.90934	0.00541	0.45967	0.48626 (0.5204)	0.11298	
2 (81 growths)	0.34484 (0.34777)	1.28406 (1.26880)	-1.21705	0.02291	0.44351	0.66475 (0.6505)	0.13434	

"Growths" which regress to zero after one step are not included. A , B and β were fitted to $\lambda + \mu/(\kappa - 1)$ using least squares; the fitted values are given in parentheses.

If $E[T_n|\kappa]$ is the expected time to n abnormal cells for a given κ , and if we conjecture that $E[1/I_n|\kappa] \leq E[1/I_n|\infty]$, then using the theory of recurrent events the ratio $E[T_n|\kappa]/E[T_n|\infty]$ can be shown to lie in the range $[1, 1 + 2/(\kappa - 1)]$. Thus the replacement of β in equation (4) by $(\text{constant}/(\kappa - 1))$ is invalid.

The parameters A , B and β in equations (3) and (4) are obviously functions of κ , the forms of which we have been unable to establish theoretically. A , B and β have, however, been fitted, using least squares, to $\lambda + \mu/\kappa - 1$ and the fitted values are given in parentheses in Table 4.

In conclusion, it seems reasonable to suppose that the growth function tends to a function of the form $A\sqrt{n}$ if large enough values of n are considered. Our results are in agreement with Williams and Bjerknes and remain so for n as large as 10,000. If, however, the "transient" part of the growth function is extracted by considering variables such as $(T_n - T_{1,600})$ for each growth, we approach the "steady state" situation and α tends to 0.5.

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Renal Blood Flow in a Diving Trained Sea Lion

THE diving reflex described for many species of animals involves a reduction in heart rate and the redistribution of blood flow to various tissues. Available evidence^{1,2} indicates that cardiac output decreases and that arterial pressure increases only modestly or remains unchanged during a dive. Flow measurements using restrained animals have tended to show cessation of flow to the kidney^{3,4} with diving. But it is well accepted that differences in these responses will exist with restrained and frightened animals as opposed to trained or free ranging animals. We investigated peripheral blood flow in a trained sea lion (*Zalophus californianus*) by measuring the renal flow

response to face immersion in water. Several months before surgery, the female sea lion was trained to place her head into a bucket, either empty or filled with water, and to remain in that position until otherwise commanded. During training, the animal wore a harness to which the necessary electronics were later attached.

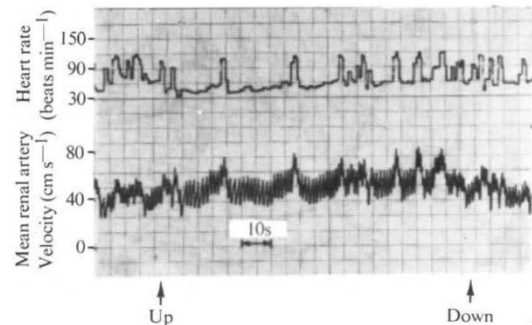


Fig. 1 Heart rate and mean renal artery flow velocity during breath holding or resting condition.

The animal was anaesthetized using a mixture of oxygen and fluothane after intramuscular injection of 0.020 mg kg⁻¹ atropine. An 8 MHz Doppler flow cuff was implanted on the left renal artery under sterile surgical conditions. Care was exercised in the dissection of the artery so that minimal damage would be done to the perivascular renal nerves. The lead wires were tunnelled under the skin to the dorsal surface of the neck at which point they were brought through the skin. The mid-line incision was closed with interrupted sutures. For the next seven days, the animal was given oral antibiotics. Training was resumed about four days after surgery, but experiments were not performed until four weeks after the operation. The Doppler flow meter used followed the design of Franklin⁵. Recordings were made of mean renal artery flow velocity and heart rate using the flow velocity pulse to trigger the cardi-tachometer. Jugular vein blood samples were obtained both pre- and post-operatively for red and white cell counts.

We show a representative portion of a control recording in Fig. 1. The resting heart rate on this occasion was 75 beats min⁻¹ and the control velocity was 45 cm s⁻¹. The "down" arrow indicates the immersion of the animal's head into an empty bucket and the "up" arrow indicates the reverse. Breath holding periods can be observed with the heart rate as

low as 36 beats min^{-1} and the mean renal artery velocity varying with the rate. Thus breath holding in this situation was part of the effect of training. The average resting heart rate was 85 beats min^{-1} and the average resting mean renal artery velocity was 48 cm s^{-1} for this animal during all of the experimental periods.

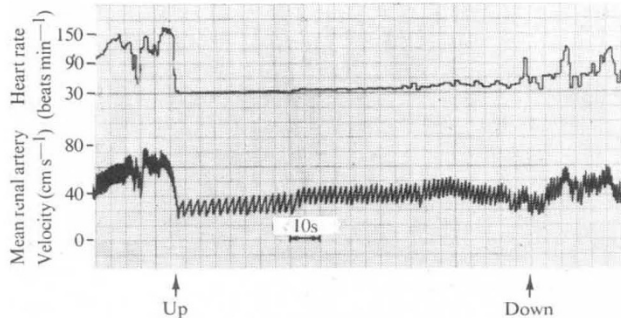


Fig. 2 Heart rate and mean renal flow velocity during face immersion for 2 min.

The record shown in Fig. 2 was obtained during an immersion in water lasting 2 min. A respiratory sinus arrhythmia occurred prior to immersion (right hand edge Fig. 2). Upon immersion a striking bradycardia began, with nadir of 30 beats min^{-1} . A reduction in mean renal artery velocity to 28 cm s^{-1} also occurred with some over-shoot in flow at the termination of the dive. The average length of 16 dives by the animal was 80 s. The mean heart rate at the end of the dive was 34 beats min^{-1} and the mean renal artery velocity was 31 cm s^{-1} , which represents an average reduction of 34% below the control value. The health status of the animal as evaluated by red and white cell counts from jugular venous blood were almost identical. Because velocity is related to volume flow by the cross-sectional area of the vessel, which is fixed in the present experiment, inferences about flow can be made.

From the studies in this unrestrained animal, it seems that inflow to the kidney does not cease but is reduced together with the cardiac output as has been shown in the duck⁶. We offer three possible explanations for the variance from previous studies: First, intrarenal redistribution of blood flow; second, species difference; third, fright. Intrarenal redistribution of flow has been demonstrated in the kidney of non-diving mammals^{7,8}. There is no way to rule out species difference; but fright is known to produce severe reaction in all animals. The record (Fig. 2) also demonstrates that there is an immediate reduction in flow, as occurs with cardiac output; thus these reductions are of neural origin. The delayed reduction in renal flow and the post-dive over-shoot of the flow response are probably related to the duration of the dive. Afferent signals of renal origin may influence the duration of diving. Therefore, oxygen may be conserved in the kidney by shunting blood around highly metabolic areas such as cortex, and simultaneously these shunts may be used to adjust vascular resistance to ensure adequate perfusion of the heart and brain and to reduce the work of the heart specifically.

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Protection against Enteric Disease caused by *Escherichia coli* – a Model for Vaccination with a Virulence Determinant?

ALTHOUGH vaccination has assisted the control of many bacterial diseases, parenteral vaccination against enteric disease is not as satisfactory as we could wish¹. This is attributable to incomplete knowledge of both the pathogenesis of intestinal infections and the protective immune responses of the alimentary tract, with the result that vaccine development has been largely empirical. New knowledge of the specific determinants of microbial pathogenicity² provides a sounder basis for the development of effective vaccines and the following report is an example of this approach to disease control.

The pathogenesis of porcine neonatal diarrhoea involves colonization of the intestine by an enteropathogenic strain of *Escherichia coli*³ followed by production of enterotoxins⁴⁻⁷. Most strains of *E. coli* that cause neonatal diarrhoea synthesize one of two closely related protein surface antigens designated K88ab or K88ac⁸⁻¹⁰. The K88 antigen has adhesive properties that seem to be essential for the virulence of K88-positive enteropathogenic strains of *E. coli*¹¹ and enable K88-positive bacteria to proliferate in the anterior small intestine¹². Thus, the two important virulence determinants of these strains of *E. coli* are (i) K88 antigen, which enables the bacteria to attach and attain large numbers in the small intestine, and (ii) enterotoxins, which cause severe diarrhoea, resulting in dehydration and death.

From these observations, we deduced that piglets could perhaps be protected against neonatal diarrhoea either by preventing bacterial attachment to the intestinal wall or by neutralizing the effects of enterotoxins. Antibodies directed against enterotoxins may be protective^{13,14}, but protection has also been observed which seemed to be antibacterial rather than anti-enterotoxic^{15,16}. Attachment of K88-positive bacteria to piglet intestinal tissue can be demonstrated *in vitro* and is inhibited by K88 antisera¹¹. Therefore, the antibacterial activity described *in vivo* could be due to an anti-K88 factor which prevents bacterial adhesion. There is no detectable placental transfer of antibodies in the pig^{17,18}; so we postulated that K88 antibodies transmitted via the colostrum to the piglet's intestinal tract should reduce attachment of K88-positive enteropathogenic bacteria to the mucosa and render the organism less virulent.

We investigated this hypothesis by immunizing pregnant gilts with partially-purified K88 antigens, then challenging their piglets with a virulent K88-positive strain of *E. coli*. K88 antigens were extracted from two laboratory-derived K88⁺ strains [08:K27(A)⁻, K88ab:H⁻ and 08:K27(A)⁻, K88ac:H⁻] prepared by introducing the plasmids which determine