Table 1 Effects of portacaval anastomosis and testosterone in rats: autopsy data

	n	Body weight (g) Initial	Autopsy	Mean change in body weight (%)	Relative organ weight (% of body weight) Liver	Kidney	Spleen	Pale cells	Zone III atrophy	Bladder calculi
Control	9	637	659	+3.6	3.46	0.68	0.25	0	0	0
PCA (P)	9	492	475*	−2.0	2.26*	0.78	0.31‡	4	3	3
Testosterone (T)	7	661	585*	−8.4	3.77	0.86†	0.22	0	0	0
PCA + testosterone (PT)	8	486	429* †(PT–T)	−11.6* †(PT–P)	2.65‡ (PT–P)	1.3 †(PT–P)	0.36‡	4	4	5

*$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$.
Weight changes are those from beginning of testosterone treatment. P values are for comparison of treatment against control unless specified (PT–T, PT–P), using two-tailed t-test.

it is known that some androgens produce adenomas in humans[2] and facilitate the development of hyperplastic nodules in carcinogen-fed rats[3], we also tested the effect of testosterone (T) on PCA rats.

Male Wistar rats (Charles River) approximately 250 g were subjected to PCA or sham operation over a $2\frac{1}{2}$-month period. Others were kept as controls. All were maintained on Purina rat chow Formulab and tap water *ad libitum* in wire bottom cages, four or five per cage. Five PCA rats killed 10 months after operation did not have nodules. Half of the remaining rats were given 200 mg testosterone enanthate (Delatestryl; Squibb) in sesame oil subcutaneously at monthly intervals for three doses. The other half received oil only (Table 1). There were six intercurrent deaths (two PCA, two PCA + T, two T); three of these animals were autopsied and found to be free of liver nodules. The remaining animals were killed one month after the last dose and $13\frac{1}{2}$–$15\frac{1}{2}$ months (mean $14\frac{1}{2}$) after operation. Under ether anaesthesia, Evan's blue in saline was injected into the spleen and the anastomosis was considered satisfactory if the liver did not darken within 10 s. Otherwise the animals were rejected from the study. Sham and control animals showed no significant difference and the data were pooled.

The livers were examined carefully for gross or microscopic nodules. None were found in any group. PCA decreased the body and liver weights and increased the spleen weights whether expressed as absolute or relative weight (% of body weight). Atrophy of zone III hepatocytes with an increased nucleo-cytoplasmic ratio in these cells compared to zone I cells was seen in 7 of 17 PCA ± T rats. Scattered hepatocytes with larger vesicular nuclei and pale cytoplasm lacking the usual coarsely granular basophilia were seen in 8 of 17 PCA ± T rats (Table 1). These cells were located in plates one cell thick and were not clustered to form nodules. The spleen in the PCA ± T groups had follicles similar in size and character to those of controls but there were more lymphocytes and occasional plasma cells in the cords of the PCA spleens. Bladder calculi, often accompanied by pyelonephritis, were common after PCA.

Testosterone caused yellowing of the fur, gross enlargement of the seminal vesicles and a decrease in the body weights. There was a relative increase in liver weight in the PCA group and absolute as well as relative increase in kidney weight in both PCA and control groups.

PCA in this study induced liver atrophy but not hyperplastic nodules. Strain and dietary differences are likely explanations for the discrepancies between this work and that of Weinbren and Washington.

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On codon usage

THE total nucleotide sequence of the *lac* repressor gene *I* (361 codons assigned) has recently been determined by Farabaugh[1]. He has found that the codon usage is quite different from that of bacteriophage MS2 and ΦX174 (1,068 and 1,346 codons assigned, respectively). Nevertheless, assuming that both the repressor protein and the phage proteins are synthesised rapidly, he concluded that the codon usage presumably does not reflect a difference in efficiency of translation. As mentioned also by Farabaugh, however, no firm data actually exist on the relative translational efficiency. Moreover, even a small effect, difficult to reveal by direct methods, can have a strong selective value when judged over many generations.

Bacteriophage MS2 produces a burst size of 5,000 to 10,000 plaque-forming units (PFU) per cell, and each virus particle contains 180 coat protein molecules. Consequently, we have argued before that MS2 has optimised translational efficiency, especially of the coat cistron: one of these ways, among others, is by the proper choice of degenerate code words[2,3]. It is likely that there exists an optimal interaction energy between the codon and the cognate anticodon. As a result, codons of the type (A/U)-(A/U)-Y—where the first and second letters give an intrinsically weaker interaction—would be stabilised if the third letter is C. Indeed, in MS_2RNA the N-N-C codon is preferred over N-N-U for all codons of this type (Phe, Ile, Tyr and Asn)[4]. Conversely, intrinsically strongly interacting codons of the type (G/C)-(G/C)-Y should be loosened by a preference for U. In fact, N-N-U codons are preferred over N-N-C for Pro, Ala and Gly (Arg codons are not included as they are recognised by an inosine-containing tRNA).

When we now consider the codon usage in the *lacI* gene, which makes the *lac* repressor protein at very low levels, we find exactly the opposite situation. U as a third letter is preferrable to C for codons of the weak interaction type (Phe, Ile, Tyr and Asn); this is remarkable, considering that of third letters 28.6% are C and only 21.1% are U. Conversely, for the codons with a strong interaction in positions 1 and 2, the third letter is preferably a C rather than a U (Pro, Ala, Gly). This selective pattern of codon usage in the two systems is very striking and it would be of considerable interest if it could be related to a difference in translational efficiency. Calos[5] has shown that the low level of *lacI* expression can be explained by a low-efficiency promoter; as pointed out by Müller-Hill *et al.*[6], one can also easily imagine that the low level of constitutive *I* expression is further reduced by some limiting step in the translation processes, such as selective codon usage.

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