the caloric density of their carcass, and show greater food-induced thermogenesis, greater noradrenaline induced thermogenesis and increased concentration of L-α-glycerophosphate oxidase in the liver. These results do not seem to be consistent with the concept that defective thermogenesis is responsible for the development of obesity. The results seem to indicate, rather, that increased intake induces obesity and that the simultaneous proportional or enhanced increase in thermogenesis is incapable of correcting the energy balance sufficiently to prevent the obesity.

This is not to say that the hypothesis proposed is unreasonable; variation in the heat increment of feeding may play a part in individual susceptibility to obesity. But we think that it should be made clear that the evidence presented and cited does not give any support to the hypothesis.

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[1] Stirling, J. L., and Stock, M. J., *Nature*, **220**, 801 (1968).
[2] Miller, D. S., and Payne, P. R., *J. Nutrit.*, **78**, 255 (1962).

MR STOCK writes: The main criticism was levied against our support for the suggestion that defective thermogenesis may be responsible for the development of obesity. It was not, however, the intention of our article to pronounce in detail on this hypothesis but rather to explain the origins of thermogenesis in the normal (non-obese) animal. The supporting evidence for the hypothesis has been given elsewhere (refs. 2, 3 and 5 in our paper) and relies to a considerable degree on the work with human subjects—work which Morrison and Millar omitted in their consideration of the theory. In defence of the hypothesis, however, I would point out that the confirmed ability of overfed lean subjects to control body weight by thermogenesis together with the evidence for defective thermogenic mechanisms in the obese provides ample grounds for suspecting a thermogenic defect in obesity. Indeed, the concept probably has better experimental verification than the more popular practice of blaming gluttony—a sin which, by common observation, appears to be practised by thin and fat alike.

Concerning our experiments, we would agree that the low protein group of rats were "relatively obese" but would point out that this was relative to a group of semi-starved controls. Similarly, in the experiments of Miller and Payne the same low protein group showed a relative obesity which was caused by a greater loss of energy in the control group. Thus what Morrison and Millar call "obese" rats are in fact normal rats that have more carcass energy than emaciated control rats.

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Attenuation of Rhinovirus Type 15 for Humans

HERE we describe results which indicate that third passage in human embryonic lung fibroblasts (WI-26) resulted in attenuation of rhinovirus type 15, and suggest that such attenuated strains might offer an approach to control of rhinovirus common colds.

Preparation and safety-testing of inocula, inoculation methods, virus isolation and identification procedures, neutralization tests and clinical evaluations were performed with reported methods[1,2]. Two stocks of rhinovirus type 15 (strain NIH 1734) were prepared for volunteer inoculations and written informed consent was obtained from inmates of the Texas Department of Correction. One stock was obtained from harvests of second passage in WI-26 cells, and one from harvests of third passage in WI-26 cells. Titres of virus were 4·5 and 5·0 $\log_{10}$ 50 per cent tissue culture infectious doses ($TCID_{50}$) per ml., respectively.

Table 1. RESPONSE OF VOLUNTEERS TO NASAL INOCULATION WITH VARYING DOSES OF RHINOVIRUS TYPE 15 (1734 WI_3)

Dose ($TCID_{50}$*)	No. of volunteers	No. infected	No. ill
32,000	1	1	0
1,000	3	3	0
10	3	3	0
1	3	1	0
0·01	4	0	0

50 per cent human infectious dose (HID_{50}) = 1·0 $TCID_{50}$ (95 per cent confidence interval 0·1, 9·6).
* 50 per cent tissue culture infectious dose.

Titration of inoculum 1734 WI_3 in volunteers who did not possess detectable serum neutralizing antibody to rhinovirus type 15 is shown in Table 1. The 50 per cent human infectious dose (HID_{50}) was estimated by the Karber method to be 1·0 $TCID_{50}$. By contrast, previous studies with inoculum 1734 WI_2 indicated an HID_{50} of 0·032 $TCID_{50}$ (refs. 3 and 4). Thus there was a thirty-fold decrease in infectivity for man associated with third passage of rhinovirus type 15 in WI-26 cells.

Table 2. COMPARISON OF VOLUNTEER RESPONSES TO TWO RHINOVIRUS INOCULA: 1734 WI_2 AND 1734 WI_3

Inoculum	No. of men with indicated response: Infected	Ill	No. of virus isolates per man (mean)		Serum neutralizing antibody titres* (reciprocal $\log_2$ mean)	
1734 WI_2	17	15†	6·9	$P < 0.01$‡	3·9	$P > 0.20$
1734 WI_3	8	0	3·1		4·1	

* All volunteers free of detectable antibody before inoculation.
† Thirteen afebrile upper respiratory illnesses (URI) and two febrile URI ($T \geq 37.7°$ C).
‡ t test.

Comparative responses of volunteers using these two inocula are shown in Table 2. Fifteen of seventeen volunteers infected with inoculum 1734 WI_2 developed illness, and two were febrile. By contrast, inoculum 1734 WI_3 has failed to produce any observable signs or symptoms in eight infected volunteers. Virus doses ranged from 0·1 to 32,000 $TCID_{50}$ for the WI_3 inoculum and were comparable with those of the WI_2 pool. Although all the inoculations reported in Table 2 were not performed simultaneously, continued ability of the WI_2 pool to produce illness when inoculated in low doses has been demonstrated during the same time period. Furthermore, the frequency of virus shedding among volunteers inoculated with 1734 WI_3 was less than among volunteers given 1734 WI_2. By contrast to these differences, in seven of eight volunteers inoculated with 1734 WI_3 and sixteen of seventeen inoculated with 1734 WI_2 serum neutralizing antibody titres increased four-fold, and mean responses were nearly identical for the two groups. The illness produced by 1734 WI_2 was similar to that observed in previous studies of sixty-four volunteers who received intranasal inoculation with second passage harvests of WI-26 cell cultures of rhinovirus types 13, 15 and 17 (refs. 2, 5 and 6). In those studies, 80 per cent of the volunteers developed typical common colds, 10 per cent had febrile colds, and 10 per cent had inapparent infections. This corresponds to findings with naturally occurring rhinovirus infections[7], and indicates that little or no attenuation of rhinoviruses occurs from two passages in WI-26 cells.

The present studies have demonstrated attenuation of a rhinovirus strain after three passages in human embryonic lung fibroblast tissue cultures. Attenuation was indicated by a thirty-fold decrease in infectivity to man,

failure to produce illness and decreased frequency of virus shedding. Serum neutralizing antibody response was not affected. These findings suggest that it may be possible to develop attenuated rhinovirus vaccines, and indicate the need for determining if attenuation of other rhinovirus serotypes occurs following a limited number of tissue culture passages.

Attenuated vaccines administered nasally may offer some promise for control of rhinovirus common colds. At present, control of rhinovirus common colds by means of inactivated vaccines appears impractical because of the large number of serological types and the lack of predominance of one or several serotypes. In addition, although monovalent inactivated vaccines have been shown to be antigenic, the serum titres of antibody achieved have been poor, and protection, as demonstrated by challenge studies, has been inconsistent[8,9]. Attenuated vaccines are usually more antigenic than their inactivated counterparts, and local infection would be more likely to induce higher levels of secretory IgA antibody which is considered very important in prevention of respiratory viral infections[10,11]. Thus homotypic protection should be optimal from attenuated vaccines. In addition, protection against subsequent rhinovirus illness not involving antibodies and lasting up to 16 weeks has been demonstrated following rhinovirus infection[6]. If this heterotypic resistance follows infection with attenuated rhinovirus, an additional advantage would ensue. Tests are currently under way to determine if infection with the attenuated strain described here is followed by homotypic and heterotypic resistance.

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[1] Knight, V., in *Progress in Medical Virology* (edit. by Barger, E., and Melnick, J. L.), **6**, 1 (S. Karger, Basel and New York, 1964).
[2] Cate, T. R., Couch, R. B., and Johnson, K. M., *J. Clin. Invest.*, **43**, 56 (1964).
[3] Couch, R. B., Cate, T. R., Douglas, jun., R. G., Gerone, P. J., and Knight, V., *Bact. Rev.*, **30**, 517 (1966).
[4] Douglas, jun., R. G., Lindgren, K. M., and Couch, R. B., *New Engl. J. Med.*, **279**, 776 (1968).
[5] Douglas, jun., R. G., Couch, R. B., Cate, T. R., Gerone, P. J., and Knight, V., *Amer. Rev. Resp. Dis.*, **94**, 159 (1966).
[6] Fleet, W. F., Couch, R. B., Cate, T. R., and Knight, V., *Amer. J. Epidemiol.*, **82**, 185 (1965).
[7] Gwaltney, J. M., and Jordan, jun., W. S., *Bact. Rev.*, **28**, 409 (1964).
[8] Mufson, M. A., Ludwig, W. M., James, H. D. T., Gould, L. W., Rourke, J. A., Holper, J. C., and Chanock, R. M., *J. Amer. Med. Assoc.*, **186**, 578 (1963).
[9] Doggett, J. E., Bynoe, M. L., and Tyrrell, D. A. J., *Brit. Med. J.*, **1**, 34 (1963).
[10] Cate, T. R., Rossen, R. D., Douglas, jun., R. G., Butler, W. T., and Couch, R. B., *Amer. J. Epidemiol.*, **84**, 352 (1966).
[11] Smith, C. B., Purcell, R. H., Bellanti, J. A., and Chanock, R. M., *New Engl. J. Med.*, **275**, 1145 (1966).

A Reappraisal of Cystic Fibrosis

Danes and Bearn[1] have reported increased metachromasia in cultured skin cells in cystic fibrosis (CF), and their observations have been confirmed recently by Matalon and Dorfman[2], who find that the acidic mucopolysaccharides are of an apparently normal type in these cells, although they are present at two to five times the usual level.

Because no clinical abnormality has hitherto been observed in mucopolysaccharide metabolism, these findings are surprising. Further complexity has been added to the cystic fibrosis picture recently by the observations of Gugler *et al.*[3] that polyacrylamide gel electrophoresis patterns of cystic fibrosis salivary proteins show abnormalities which can be removed by the addition of EDTA; calcium ions have been implicated as the cause of the abnormalities.

I believe that these observations, together with the usual clinical findings in cystic fibrosis—disordered epithelial glycoprotein secretion and high concentrations of exocrine Na^+ ions—can be reconciled in terms of a single, generalized, inheritable defect in cellular Na^+ ion uptake, for which there is now evidence in sweat glands[4], in submaxillary glands[5] and in red cells[6].

The secretion of glycoproteins or acid mucopolysaccharides involves essentially the movement of highly negatively charged molecules from the cell. To maintain overall electrical neutrality, cations, most likely Na^+ ions, must leave the cell simultaneously. With a reduced capacity to take up Na^+ ions, secretory activity might be impaired in three ways: (*a*) secretory materials may build up in the cells concerned; (*b*) other ions, such as calcium ions, may serve as the accompanying cation; (*c*) the molecules secreted may become less negatively charged, for example, by the substitution of fucose (neutral) for neuraminic acids (charged) in epithelial glycoproteins.

The first impairment is a common pathological condition of the goblet cells of the gastro-intestinal tract in cystic fibrosis, the second seems to account for the salivary electrophoresis results and the third was noted by Dische *et al.* as long ago as 1959 (ref. 7).

Work with cystic fibrosis skin fibroblasts suggests that, in culture, restriction of acid mucopolysaccharide secretion occurs and secretory materials accumulate. Acid mucopolysaccharide production by cells *in vitro* seems sufficiently rapid and uncontrolled for this to occur, a situation which does not appear to be duplicated *in vivo*, perhaps because acid mucopolysaccharide production is slowed to the point where secretion of cations can keep up with it, despite defective uptake of Na^+ ions. It is significant that abnormalities of epithelial glycoprotein secretion occur in cystic fibrosis, a process which can, on occasion, be very rapid, and moreover takes place in a predominantly hypotonic environment, which should aggravate any defect in Na^+ ion uptake.

This theory also offers an explanation for the impairment of pancreatic bicarbonate secretion in cystic fibrosis, which formed the basis of a recent speculative article[8]. Bicarbonate is negatively charged and cells involved in its active secretion would need to release positively charged species simultaneously. Failure to retain sufficient positive charges could lead to the pancreatic dysfunction described.

I believe that a number of the apparently unconnected findings relating to cystic fibrosis appear to be reconciled on the basis of a shortage of intracellular sodium in secretory cells.

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[1] Danes, B. S., and Bearn, A. G., *Lancet*, **i**, 1061 (1968).
[2] Matalon, R., and Dorfman, A., *Biochem. Biophys. Res. Commun.*, **33**, 954 (1968).
[3] Gugler, E., Pallavicini, C. J., Swerdlow, H., and di Sant'Agnese, P. A., *J. Pediat.*, **71**, 585 (1967).
[4] Emrich, H. M., Stoll, E., Friolet, B., Colombo, J. P., Rossi, E., and Richterich, R., *Bibliotheca Paediatrica*, **86**, 58 (1967).
[5] Mandel, I. D., Kutscher, A., Denning, C. R., Thompson, R. H., and Zegarelli, E. V., *Amer. J. Dis. Child.*, **113**, 431 (1967.
[6] Balfe, J. W., Cole, C., and Welt, L. G., *Science*, **162**, 689 (1968).
[7] Dische, Z., di Sant'Agnese, P. A., Pallavicini, C., and Youlos, J., *Pediatrics*, **24**, 74 (1959).
[8] Johansen, P. G., Anderson, C. M., and Hadorn, B., *Lancet*, **i**, 455 (1968).