Table 3. Summary of the approximate frequencies (percentages) of Gm and Inv factors in chimpanzees and various races of man.

Population*	Gm factors							Inv factors	
	a	x	b^1	b^2	b^3	b^4	c	l	b
White (U.S.)	57	20	90	90	90	90	0	20	99
Negroid (African)	100	0	100	0	90	95	30–100	50	90
Mongoloid (Japanese)	100	30	20	20	60	20	0	50	88
Chimpanzee	100	0	0	0	100	100	35	25	?

* The data on White, Negroid, and Mongoloid populations are taken from Steinberg and co-workers (*12, 14, 18*).

Gm(c). Chimpanzees differ from Negro populations in being 100 percent Gm(b^1—).

A further similarity between the Gm factors of man and chimpanzee is their mode of inheritance. In man, Gm factors are inherited as dominants or codominants (*10*). Studies of four chimpanzee families containing both parents and one or more offspring indicate that Gm(c), the only Gm factor showing polymorphism in the chimpanzee is inherited as a dominant in this species.

The amount of γ-globulin as determined by the zinc turbidity method (*16*) in 64 of the chimpanzee serum samples ranged from 0.64 to 1.38 g/100 ml. This compares with the range of 0.71 to 1.3 g/100 ml for normal human serums. No correlation was observed between the amounts of IgG and Gm or Inv types. The absence of Gm(b^1), and Gm(b^2), and Gm(x) and the variable presence of Gm(c), Inv(l) and Inv(b) in the chimpanzee do not merely reflect differences in serum γ-globulin concentrations.

Chimpanzee serums were tested by Ouchterlony analysis with monkey antiserums specific for human γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins (*2*). All serums tested gave precipitin bands with each antiserum. Similarly, Ouchterlony tests with rabbit antiserums specific for either κ- or λ-chains were positive for all tested serums. Hence, chimpanzee serums contain molecules antigenically related to human γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins, and also to human κ- and λ-chains.

Thus chimpanzee serums contain at least four of the human Gm factors and two Inv factors. They also contain molecules antigenically related to those heavy and light polypeptide chains of human IgG that seem to be necessary substrates for the expression of Gm and Inv factors. Whole serum was used in these experiments, and it will be important in the future to isolate immunoglobulins from chimpanzee serums and examine genetic and antigenic characteristics of the isolated proteins. In addition, since there may be subspecies or races of chimpanzees (*17*), a more complete understanding of primate immunoglobulin genetics will probably require investigation of subspecies of chimpanzees, as well as of other nonhuman primates.

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Active Transport of 5,5-Dimethyl-2,4-Oxazolidinedione

Abstract. *5,5-Dimethyl-2,4-oxazolidinedinedione, a substance commonly used to estimate intracellular* p*H, moves against both a concentration gradient and a hydrogen-ion gradient in the everted gut sac. Furthermore, the value of the flux ratio for this substance under conditions of zero electrochemical potential across the bowel wall unequivocally demonstrates active transport.*

In recent years partitioning of the weak acid 5,5-dimethyl-2,4-oxazolidinedione (DMO) between the intra- and extracellular fluid has been used to estimate intracellular *p*H (*1*). Implicit in the method are the essential assumptions (i) that the acid is not bound to intra- or extracellular proteins, (ii) that its disassociation constant (pK_a) is the same inside and outside the cell, (iii) that, essentially, it is not metabolized, and finally (iv) that it is passively distributed between the intra- and extracellular fluid in accordance with the hydrogen-ion gradient across the cell membrane. If any one of these assumptions is incorrect in any biologic system, then the uncritical use of DMO in other systems may be invalid. While several studies have shown that DMO is neither metabolized nor bound to protein (*2*), the possibility that it is actively transported in mammalian tissue has never been carefully investigated. Should there exist, for example, a carrier mechanism in the plasma membrane for the transport of DMO into the cell against an electrochemical gradient, one would expect an intracellular concentration higher than that appropriate for the actual H^+ gradient; hence the calculated value for the intracellular *p*H would be erroneously high. The small intestine of the rat, a tissue that actively transports a number of substances, was used in our investigation because experimental methods were available which provided rigid control of transmembrane potential differences as well as concentration and *p*H gradients.

Sprague-Dawley female rats (240 to 260 g) were given free access to rat chow and water before they were killed by decapitation. The entire small bowel was excised, flushed of its contents with cold saline, and cut into ten segments of equal length. For purposes of identification these segments were numbered 1 through 10, from the

proximal to the distal end; they were then used for preparation of everted gut sacs or were placed in an in vitro perfusion apparatus (*3*) which permitted unidirectional flux rates to be determined at zero electrochemical potential across the intestinal wall. The C^{14}-labeled DMO (5,5-dimethyl-2,4-oxazolidinedione-2-C^{14}) was shown to be pure by gas-liquid chromatography; a second compound used in the experiments, α-aminoisobutyric-1-C^{14} acid (AIB), was not checked by chromatography (*4*).

Everted gut sacs were placed in Krebs bicarbonate buffer so that 1 ml of solution was bathing the serosa and 10 ml was bathing the mucosa; the two solutions initially contained concentrations of either C^{14}-labeled DMO or AIB equal to 77 nmole/ml. After a 90-minute incubation period at 37°C in a metabolic shaker, fluid from both compartments was assayed for C^{14} content in a Packard liquid scintillation counter; results are expressed in Fig. 1 as "serosal-mucosal ratios"—that is, ratios between the C^{14} activities, in counts per minute per milliliter of perfusate, in serosal fluid and mucosal fluid.

In experiment A1, in which no glucose was present in the buffer, DMO was clearly transported against a concentration gradient by the mid-intestinal segments. Although the initial *p*H in both compartments was 7.40, at the end of the incubation period the *p*H of the serosal fluid invariably dropped into the range of 6.55 to 7.00 and that of the mucosal fluid rose to 7.45 to 7.50; thus in this study DMO was not only being moved against a concentration gradient but, just as importantly, it was also being transported against an H^+ gradient. However, as shown in experiment A2, even in the presence of a favorable *p*H gradient the nonionic distribution of DMO across the gut wall did not significantly enhance the observed serosal-mucosal ratios. Finally, the highest ratios, greater than 3.0, were obtained when glucose in a concentration of 200 mg/100 ml was added to both the mucosal and serosal fluids (experiment A3).

For purposes of comparison the behavior of the nonmetabolizable, actively transported amino acid AIB (*5*) was studied in the same three experimental situations. While the values for the serosal-mucosal ratios of AIB were independent of the presence of glucose in the media (experiments B1 and B3) or alterations in the *p*H gradient (experiment B2), what is of particular importance in these studies is the finding that, in the presence of glucose, serosal-mucosal ratios were obtained with DMO which were fully as high as those obtained with the actively transported AIB; these data, therefore, strongly suggest that DMO is also actively transported by mid-intestinal segments.

In order to better validate this possibility the magnitude of the bidirectional flux rates of DMO across the

Table 1. Bidirectional flux rates for DMO and AIB in segments of rat intestine. In these studies the transmural potential difference was maintained at zero by means of a short-circuiting apparatus. The concentration of DMO or AIB was initially 77 nmole/ml in both the serosal and mucosal fluids; the concentration did not change detectably during the experiment because the volume of fluid was large. The *p*H of both fluids also remained fixed, at 7.40 ± 0.05, throughout each experiment. Unidirectional flux rates were determined by adding isotopic amounts of C^{14}-labeled DMO or AIB to either the serosal or mucosal solution and following the appearance of labeled material in the other solution. The mucosal-to-serosal (M_{m-s}) and serosal-to-mucosal (M_{s-m}) flux rates are shown along with the flux ratios (M_{m-s}/M_{s-m}); in each of these experiments the two unidirectional flux rates were individually measured in two adjacent bowel segments. Data on the net movement of water from mucosal solution to serosal solution are also given; a negative value indicates net movement in the opposite direction.

Expt. No.	Substance tested	Mannitol added (mmole liter^{-1})	Flux: Rate (pmole min^{-1}cm^{-1}) M_{m-s}	Flux: Rate (pmole min^{-1}cm^{-1}) M_{s-m}	Ratio (M_{m-s}: M_{s-m})	Water movement (μl min^{-1}cm^{-1})
Krebs buffer in both compartments (pH 7.4)						
C1	DMO	0	105	43	2.4	1.38
C2	DMO	0	94	46	2.0	1.00
Glucose (200 mg/100ml) in both solutions; mannitol in mucosal solution alone						
D1	DMO	20	335	41	8.2	0.92
D2	DMO	30	235	61	3.9	0.90
D3	DMO	40	393	94	4.2	−0.04
E1	AIB	40	347	84	4.1	0.20
E2	AIB	40	295	37	7.9	−0.15

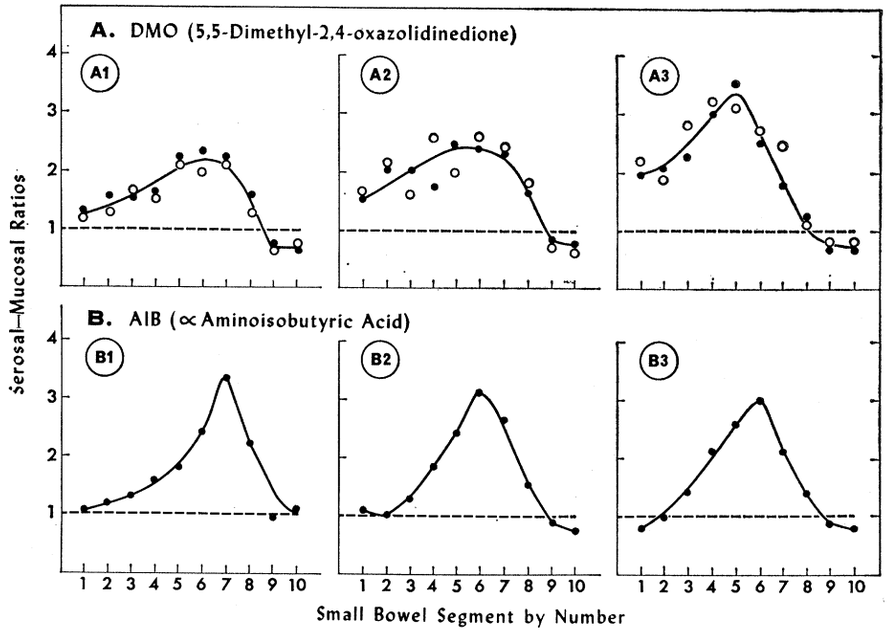

Fig. 1. DMO and AIB transport in the everted gut sac. The location of each gut sac along the length of the small intestine is indicated by number along the horizontal axis, and the serosal-mucosal ratios developed for each of the test substances at the end of the 90-minute test period are shown on the vertical axis. In experiments A1 and B1 the mucosal and serosal solutions were both Krebs bicarbonate buffer (KBB); in experiments 2A and 2B the mucosal solution was KBB while the serosal solution consisted of an isosmotic solution of tris (O.1*M*) and saline at *p*H 7.8; in experiments 3A and 3B both solutions were KBB and glucose (200 mg/100 ml). During the incubation the *p*H of the mucosal fluid usually rose slightly (to 7.45 to 7.50) and that of the serosal fluid dropped (into the range of 6.55 to 7.00) in experiments 1 and 3; in experiment 2 it remained fixed (at 7.80 ± 0.05). The open and closed circles in experiment A represent values obtained with gut sacs from two different animals.

intestinal wall was determined in an in vitro perfusion apparatus under experimental conditions in which (i) the potential difference across the wall was short-circuited, (ii) the DMO activity was kept equal on both sides of the gut wall, and (iii) the *p*H of both the mucosal and serosal Krebs buffer remained fixed at 7.40 ± 0.05 throughout the incubation. In experiments C1 and C2 (Table 1) the flux of DMO from the mucosal to the serosal fluid ($M_{m\text{-}s}^{DMO}$) exceeded the flux in the opposite direction ($M_{s\text{-}m}^{DMO}$) by 2.4- and 2.0-fold, respectively. Addition of glucose (200 mg/100 ml) to both solutions increased this difference (experiment D). However, in these experiments there was net movement of water from mucosal fluid to serosal fluid; hence the possibility existed that the inequality of the two unidirectional fluxes was due to solvent drag and not to transport mediated by a carrier. To eliminate this possibility, increasing amounts of mannitol were added to the mucosal buffer (experiments D1, D2, and D3) until net water movement was actually reversed (experiment D3). In this situation $M_{m\text{-}s}^{DMO}$ still was 4.2-fold greater than $M_{s\text{-}m}^{DMO}$. This finding provides unequivocal evidence that DMO is actively transported by mid-intestinal segments. Again, for comparison, the flux ratios of AIB under experimental conditions in which net water flux was essentially zero are shown in experiments E1 and E2. As is apparent, both the values for $M_{m\text{-}s}^{AIB}$ and the flux ratios obtained with this actively transported amino acid are quantitatively similar to those obtained with DMO.

Thus DMO, like AIB, is actively transported in the intestine. This finding is contrary to the currently held view that DMO is distributed across biologic membranes solely by passive diffusion. However, it should be emphasized that, to our knowledge, the only experimental substantiation of this concept is the data of Robin (*6*); these data show that passive distribution of DMO, in accordance with the existing *p*H gradient, occurs across the pericardial and coelomic membranes of the spiny dogfish and semiaquatic turtle. Of greater importance, however, is the present unequivocal demonstration that DMO is actively transported in the mammalian intestine.

Many substances are actively transported by both the intestinal mucosa and the plasma membrane of the muscle cell. One is AIB, which moves against an electrochemical gradient both in the intestine (*7*) and in skeletal muscle (*5*). Should DMO also be actively transported across the plasma membrane of the muscle cell, then the calculated intracellular *p*H would be falsely high if the direction of transport were into the cell or, conversely, falsely low if the direction of transport were out of the cell.

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Adenovirus Multiplication: Genetic Relatedness of Tumorigenic Human Adenovirus Types 7, 12, and 18

Abstract. *Hybridization measurements among the DNA's of six weakly tumorigenic strains of human adenovirus type 7 reveal that all six strains are very closely related (83- to 110-percent nucleotide sequence homology). The DNA homology between these six type 7 strains and nontumorigenic types 2 and 4 is 32 percent and 52 percent, respectively. The 10- to 20-percent homology between the type 7 DNA's and those of "potent" tumorigenic types 12 and 18 indicates a very low degree of genetic relatedness. These data indicate that, if the carcinogenic potential of adenovirus types 7, 12, and 18 lies in the possession of nucleotide sequences common to these three viruses, these regions represent a small portion of the total genome, approximately one to three viral cistrons.*

Several strains (*1*) of human adenovirus type 7 have been demonstrated (*2*) to be weakly tumorigenic, that is, they produce tumors in a small proportion of animals injected with large inocula and only after a considerable period of time. Accordingly, we are investigating the degree of genetic relatedness between these strains and those of highly tumorigenic human adenovirus types 12 and 18. The degree of genetic relatedness among the six type 7 strains and between these strains and highly oncogenic types 12 and 18 and nontumorigenic types 2 and 4 was determined by DNA homology measurements with the DNA-agar technique (*3*) as used in our laboratory (*4*). DNA homology here refers to the possession by DNA molecules of nucleotide sequences sufficiently similar to be detected as DNA-DNA hybrids.

Radioactive virus was prepared by the addition of P^{32} at the time of infection of continuously cultured KB cells (established human cell line) with adenovirus or by the addition of H^3-labeled thymidine at 17 to 19 hours after infection. After the virus was harvested and purified (*5*), its DNA was extracted according to the procedure of Green and Piña (*6*) with the modifications described by Lacy and Green (*4*). The radioactive DNA was sheared in a Raytheon 10-kcy sonic oscillator to yield DNA fragments having a molecular weight of about 7×10^5 (*7*). These labeled DNA fragments were then denatured and incubated with appropriate amounts of DNA-agar; the extent of hybridization was then determined (*3*). Unlabeled DNA was extracted in the same manner and, after heat-denaturation, was trapped in agar. The amount of DNA embedded was determined by measuring the ultraviolet absorbance at 260 mμ of a portion of the DNA-agar dissolved in hot 5*M* sodium perchlorate.

From the results of the homology measurements (Table 1), all six strains of adenovirus type 7 studied are close to 100-percent related. The 83- to 110-percent homology observed with the various strains is not considered different from 100 percent in view of the experimental error (5 to 15 per-