Experimental Diabetes Reduces Circulating 1,25-Dihydroxyvitamin D in the Rat

Abstract. *Duodenal calcium absorption and a vitamin D–dependent duodenal calcium-binding protein are depressed in rats with alloxan- or streptozotocin-induced diabetes. To test for possible abnormal vitamin D metabolism in diabetes we measured serum concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in control, streptozotocin diabetic, and insulin-treated diabetic rats. The serum concentration of 1,25-dihydroxyvitamin D was depressed in untreated diabetic rats to one-eighth of the level in controls and was restored to control levels by insulin treatment. The serum concentration of 25-hydroxyvitamin D was the same in all three groups. Hence, effects of diabetes on duodenal calcium transport can be explained by reduced concentrations of 1,25-dihydroxyvitamin D resulting either from failure of renal 1α-hydroxylation of 25-hydroxyvitamin D or increased catabolism of 1,25-dihydroxyvitamin D.*

Uncontrolled experimental diabetes (induced by alloxan and streptozotocin) in the rat is associated with depressed duodenal calcium absorption and calcium-binding protein (*1*). Intestinal calcium absorption is regulated by 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], the kidney metabolite of vitamin D that acts directly on the mucosal epithelial cell (*2*). Correction of subnormal duodenal calcium absorption in diabetic rats can be achieved by treatment with 1,25-$(OH)_2$D and its synthetic precursor, 1α-hydroxyvitamin D [1α-(OH)D], but not by either vitamin D or its intermediary metabolite, 25-hydroxyvitamin D [25-(OH)D] (*3*). Alleviation of depressed calcium absorption is also effected by an extract of the South American plant *Solanum malacoxylon* (*4*), which has recently been shown to contain a glycoside of 1,25-$(OH)_2D_3$ (*5*). These data suggest a lesion in vitamin D metabolism in diabetes, perhaps in the renal 1α-hydroxylation step. Furthermore, it has been reported that in humans with diabetes there is a significant loss of bone mass whether the onset of the disease occurs in the juvenile or the adult (*6*). Since 1,25-$(OH)_2$D is considered to be the active form of vitamin D at the bone (*7*), as well as the intestine, it was of interest to seek direct evidence for abnormal vitamin D metabolism in diabetes by measuring circulating concentrations of 25-(OH)D and 1,25-$(OH)_2$D in diabetic rats.

Male albino rats of the Carworth strain weighing about 200 g were divided randomly into two groups. Rats to be made diabetic received an intraperitoneal injection of streptozotocin (Upjohn) in citrate buffer (*p*H 4.5); 100 mg/kg and 25 mg/kg were administered on two successive days, each dose in 0.5 ml of buffer. Control rats received buffer only. Criteria for diabetes were growth failure and greater than 2 percent urinary glucose concentrations. On day 3 after injection, the diabetic group was subdivided into two groups. One group of diabetics was injected with 6 international units of porcine insulin (NPH, 40 unit/ml; Eli Lilly) for the remainder of the study, while the other group of diabetics and the controls received distilled water. All rats were placed in individual stainless steel metabolism cages and given free access to commercial laboratory chow containing adequate amounts of vitamin D (1.5 unit/g, 2 percent Ca, 0.9 percent P; Teklad Mills). Food and water intake, urine volume, and urinary glucose concentrations were measured daily. On day 7 of insulin treatment (10 days after injection), control, diabetic, and insulin-treated diabetic rats were anesthetized by an intraperitoneal injection of Nembutal (35 mg/kg).

The abdominal wall was opened by a midline incision and all rats were exsanguinated from the bifurcation of the aorta with a No. 19 butterfly needle. Serum glucose (*8*), calcium (by means of atomic absorption spectrometry), and phosphorus (*9*) concentrations were determined on each sample. For vitamin D metabolite determinations, serum samples from each control, diabetic, and insulin-treated diabetic group were pooled randomly to produce five samples per group, each of which contained the serum of four or five rats. Serum concentrations of 25-(OH)D and 1,25-$(OH)_2$D were determined by the radioactive ligand receptor assay described by Hughes *et al.* (*10*). Serum (approximately 15 ml) was utilized for triplicate assay of each metabolite. After the addition of 1000 count/min each of tritiated 25-$(OH)D_3$ and tritiated 1,25-$(OH)_2D_3$ (6 to 8 c/mmole), the sterols were extracted and isolated by way of a dual three-step chromatography procedure (Sephadex LH-20, silicic acid, and micro-Celite) as detailed elsewhere (*10*). Final yields ranged from 50 to 75 percent. Although with these chromatographic procedures the 25-(OH)D and 1,25-$(OH)_2$D are processed separately, their respective D_3 and D_2 forms are not resolved. Assays were carried out by the competitive binding technique, the chick intestinal receptor system being used as described by Brumbaugh *et al.* (*11*). This receptor has an equal affinity for binding 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_2$, and both 25-$(OH)D_3$ and 25-$(OH)D_2$ compete comparably but at 500-fold higher concentrations; therefore the assay measures total 1,25-$(OH)_2$D and 25-(OH)D (*10*). The minimum sensitivity of the assay is 17 pg for 1,25-$(OH)_2$D and 10 ng for 25-(OH)D, and triplicate assays result in a 10 to 15 percent interassay variation. Preparation of the intestinal receptor system and the binding assay have been modified (*12*) since the original reports of this method (*10, 11*).

The results (see Table 1) are expressed as means ± one standard error. One-way analysis of variance followed by the Tukey's multiple comparison test was used for determination of statistical significance (*13*). In contrast to the normal growth pattern observed for controls, diabetic rats exhibited weight loss (Table 1, day 3). Diabetic rats treated with insulin, however, began to grow at a rate greater than that of controls and by day 7 of treatment weighed the same as control rats (Table 1, day 10). Food intake was slightly greater in the diabetic (34 ± 1 g/day) and insulin-treated diabetic (33 ± 1 g/day) groups than in the control group (29 ± 1 g/day). Glycosuria (> 2 percent) and hyperglycemia of the diabetic group were corrected by insulin. Of particular interest is the finding (Table 1) that serum concentrations of 1,25-$(OH)_2$D (*14*) were strikingly lower in diabetic rats than in controls and were restored to the normal level by insulin treatment. Since serum concentrations of 25-(OH)D were the same in all groups, the data on vitamin D metabolites in Table 1 are consistent with an aberration in the renal 1α-hydroxylation of 25-(OH)D in diabetes. Serum concentrations of calcium were similar in control (10.3 ± 0.1 mg/dl), diabetic (10.2 ± 0.2 mg/dl), and insulin-treated diabetic (10.2 ± 0.1 mg/dl) groups. Serum phosphorus was also similar in control (7.2 ± 0.2 mg/dl), diabetic (7.6 ± 0.5 mg/dl), and insulin-treated diabetic (6.9 ± 0.2 mg/dl) groups.

Our data indicate an abnormality in vitamin D metabolism in uncontrolled diabetes in the rat. Previous studies showed that depressed duodenal calcium absorption could be corrected by treatment

with 1,25-$(OH)_2$D, 1α-(OH)D, *S. malacoxylon* extract, or insulin (*3, 4, 15*). Neither 25-(OH)D nor vitamin D produced a transport effect in diabetes (*3*). These studies imply a defect in the 1α-hydroxylation step of vitamin D metabolism. The 1,25-$(OH)_2D_3$-glycoside of *S. malacoxylon* is glycolytically cleaved to yield the active sterol in vivo (*16*), and 1α-(OH)D requires hydroxylation by the liver for activity (*17*); therefore, both compounds are active in repairing depressed calcium absorption in diabetes without the requirement for renal metabolism to produce 1,25-$(OH)_2$D, which acts directly on the intestinal mucosal cell. Our study demonstrates that correction by insulin of calcium absorption in diabetes (*15*) is through restoration of depressed 1,25-$(OH)_2$D.

That the untreated diabetic rat is stressed with respect to maintenance of calcium homeostasis is consistent with the finding that the concentration of serum immunoreactive parathyroid hormone (IPTH) in diabetics increases to double that in controls (*18*). The increased concentration of IPTH is most probably secondary to calcium malabsorption resulting from the decreased concentrations of 1,25-$(OH)_2$D. The fact that serum calcium is not depressed in diabetes is most probably the result of this compensation by increased parathyroid function, rather than mobilization of calcium from bone by the normal levels of 25-(OH)D. Because parathyroid hormone promotes 1α-hydroxylation of 25-(OH)D, the depressed 1,25-$(OH)_2$D in the face of increased IPTH rules out parathyroid insufficiency as the cause for the decrease in 1,25-$(OH)_2$D.

The etiology of abnormal vitamin D metabolism in diabetes, which results in decreased serum concentrations of 1,25-$(OH)_2$D, is unknown. Our study shows that hepatic 25-hydroxylation of vitamin D is not affected by the disease. Because 1,25-$(OH)_2$D concentrations are very low, depressed 1α-hydroxylation by the kidney or increased destruction of 1,25-$(OH)_2$D would explain the findings. Long-standing diabetes (6 months) induced by streptozotocin in the rat is associated with a nephropathy that is the result of the diabetic state rather than the drug per se (*19*). Our data at 10 days were obtained prior to these changes; we found that the serum concentration of creatinine was normal and that the concentration of urea nitrogen in the serum was only slightly increased even after feeding (*15*). Therefore, it is unlikely that the depressed 1,25-$(OH)_2$D in our experiments was the result of renal impairment, particularly in view of the reversal of the depression that was brought about by insulin. On the other hand, it is possible that insulin itself acts directly at the kidney to maintain and modulate the 1α-hydroxylase enzyme. Because 1α-hydroxylase cannot be assayed in the rat (*20*), further kinetic studies on the turnover and utilization of 1,25-$(OH)_2$D in the diabetic state must be conducted before one of these two alternative explanations, depressed 1α-hydroxylation or increased destruction, can be eliminated.

Table 1. The body weights and concentrations of glucose, 25-(OH)D, and 1,25-$(OH)_2$D in the serums of matched control, diabetic, and insulin-treated diabetic rats. The data are expressed as means ± one standard error.

No. of rats	Body weight (g)			Serum		
	Initial	Day 3	Day 10	Glucose (mg/dl)	25-(OH)D* (ng/ml)	1,25-$(OH)_2$D† (ng/dl)
			Control rats			
25	225 ± 3	248 ± 3	285 ± 4	200 ± 3	8.7 ± 0.8	6.7 ± 0.5
			Diabetic rats			
24	232 ± 2	208 ± 3‡	201 ± 5‡§	679 ± 24‡§	8.3 ± 0.8	0.8 ± 0.2‡§
			Insulin-treated diabetic rats			
24	227 ± 3	205 ± 3‡	284 ± 3	81 ± 8‡	8.9 ± 0.8	6.0 ± 0.9

*Mean of four analyses in triplicate; for each analysis serums from four or five rats were pooled. †Mean of five analyses in triplicate; for each analysis serums from four or five rats were pooled. ‡Differs significantly from control at $P < .05$ level. §Differs significantly from insulin-treated diabetic at $P < .05$.

Several other explanations for the reduction in 1,25-$(OH)_2$D concentrations in the diabetic rat should be considered. First, because circulating 1,25-$(OH)_2$D is transported by a binding protein (*21*), proteinuria in the diabetic rat could decrease the concentrations of 1,25-$(OH)_2$D in the serum. However, proteinuria in the diabetic rat is mild even after 9 months of diabetes (*19*). In addition, since 25-(OH)D and 1,25-$(OH)_2$D are both transported by the same carrier protein (*21*), which is ordinarily only 1 to 3 percent saturated with ligand (*22*), and since 25-(OH)D levels are normal in diabetes, the greatly depressed 1,25-$(OH)_2$D levels are unlikely to be caused by depletion of binding protein. Second, since rats with diabetes induced by streptozotocin are not acidotic (*23*), no acid-base imbalance that might alter the formation or metabolism of 1,25-$(OH)_2$D is present. Third, the short half-life of streptozotocin rules out a direct effect of the drug on the 1α-hydroxylase at the time of our study. Streptozotocin toxicity would be unlikely to be reversed by treatment of the rat with insulin. Finally, diabetic rats also have adrenal hyperfunction (*24*) and this might alter vitamin D metabolism because glucocorticoids depress calcium absorption (*25*). However, since glucocorticoid treatment increases the serum concentration of 1,25-$(OH)_2$D in the rat (*26*) and in man (*27*), the low levels of 1,25-$(OH)_2$D are unlikely to be related to adrenal hyperfunction. In addition, cortisone treatment of the rat does not alter the conversion of 25-(OH)D to the 1,25-dihydroxy metabolite or the localization of the metabolite in the mucosa (*28*).

Alteration of vitamin D metabolism in uncontrolled diabetes in the rat can be extrapolated to the diabetic condition in humans only with caution. However, there are several observations that warrant consideration in this regard. For example, bone formation has been shown to be decreased in human diabetes (*29*). In addition, human diabetics of both sexes have significant osteoporosis when compared with age-matched controls (*6, 30*), and they exhibit clinical complications of osteoporosis such as vertebral body and femoral neck fractures (*31*). Vitamin D metabolism should therefore be assessed in human diabetes, especially in view of the decreased circulating concentrations of the hormonal form of the vitamin here reported for a diabetic animal model.

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References and Notes

1. L. E. Schneider and H. P. Schedl, *Am. J. Physiol.* **223**, 1319 (1972); L. E. Schneider, H. D. Wilson, H. P. Schedl, *Nature (London)* **245**, 327 (1973); *Am. J. Physiol.* **227**, 832 (1974).
2. M. R. Haussler, *Nutr. Rev.* **32**, 257 (1974); H. F. DeLuca, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 2211 (1974); A. W. Norman and H. Henry, *Recent Prog. Horm. Res.* **30**, 431 (1974).
3. L. E. Schneider, J. L. Omdahl, H. P. Schedl, *Endocrinology* **99**, 793 (1976).

4. L. E. Schneider, R. H. Wasserman, H. P. Schedl, *ibid.* **97**, 649 (1975).
5. M. R. Haussler, R. H. Wasserman, T. A. McCain, M. Peterlik, K. M. Bursac, M. R. Hughes, *Life Sci.* **18**, 1049 (1976); R. H. Wasserman, J. D. Henion, M. R. Haussler, T. A. McCain, *Science* **194**, 853 (1976).
6. M. E. Levin, V. C. Boisseau, L. V. Avioli, *New Engl. J. Med.* **294**, 241 (1976).
7. L. G. Raisz, C. C. Trummel, M. F. Holick, H. F. DeLuca, *Science* **175**, 768 (1972); R. G. Wong, A. W. Norman, C. R. Reddy, J. W. Coburn, *J. Clin. Invest.* **51**, 1287 (1972).
8. M. J. Somogyi, *J. Biol. Chem.* **195**, 19 (1951).
9. C. H. Fiske and Y. SubbaRow, *ibid.* **66**, 375 (1925).
10. M. R. Hughes, D. J. Baylink, P. G. Jones, M. R. Haussler, *J. Clin. Invest.* **58**, 61 (1976).
11. P. F. Brumbaugh, D. H. Haussler, R. Bressler, M. R. Haussler, *Science* **183**, 1089 (1974); P. F. Brumbaugh, D. H. Haussler, K. M. Bursac, M. R. Haussler, *Biochemistry* **13**, 4091 (1974).
12. Intestinal mucosa (2 g) from one rachitic chick is homogenized in 25 ml of 0.25*M* sucrose in 0.05*M* tris-HCl (*p*H 7.4), 0.25*M* KCl, and 0.005*M* $MgCl_2$. The cytosol fraction is obtained by centrifugation at 100,000*g* for 1 hour. Chromatin is prepared from crude nuclei (isolated from the original homogenate by centrifugation at 1000*g* for 10 minutes) by homogenizing them successively in one 25-ml portion of 0.8 m*M* EDTA, 25 m*M* NaCl, *p*H 8; one 25-ml portion of 1 percent Triton X-100, 0.01*M* tris-HCl, *p*H 7.5; and one 25-ml portion of 0.01*M* tris-HCl, *p*H 7.5. The chromatin is harvested by sedimentation at 30,000*g* for 10 minutes after each wash. The entire chromatin pellet from 2 g of mucosa is reconstituted with half the cytosol fraction by homogenization to create a cytosol-chromatin receptor system for the competitive binding assay. The reconstituted homogenate is then forced through a 22-gauge needle. All operations are performed at 0 to 4°C, and receptor system is prepared immediately prior to use. To each assay tube containing tritiated 1,25-$(OH)_2D_3$ and unlabeled sterol (dried together with a stream of nitrogen) is added 10 μl of distilled ethanol and 100 μl of the reconstituted cytosol-chromatin system (containing about 100 μg of DNA). The final concentration of tritiated 1,25-$(OH)_2D_3$ is 4.3 n*M*. After incubation for 30 minutes at 25°C with vigorous shaking in a water bath, the quantity of labeled sterol bound to chromatin is determined by filtration. To each assay tube, 1 ml of cold 1 percent Triton X-100 in 0.01*M* tris, *p*H 7.5, is added and the entire mixture applied to a Gelman Type A/E glass fiber filter at very low vacuum. After 2 to 4 minutes the vacuum is increased to achieve uniform flow rates of about 1 ml per minute, and each of the filters is washed with 2 ml of 1 percent Triton X-100 and 0.01*M* tris, *p*H 7.5. After filtration, the filters are placed in liquid scintillation vials with 5 ml of methanol-chloroform mixture (2 : 1 by volume). After 20 minutes the methanol-chloroform mixture is evaporated and the sterols are solubilized in a standard toluene base mixture and counted according to liquid scintillation procedures. Sterol sources were as follows: nonradioactive 25-$(OH)D_3$, [Dr. J. Hinman] Upjohn; tritiated 25-$(OH)D_3$ (6 to 8 c/mmole), Amersham/Searle; nonradioactive 1,25-$(OH)_2D_3$, [Dr. M. Uskokovic] Hoffman La Roche; the tritiated 1,25-$(OH)_2D_3$ was generated enzymatically from 25-$(OH)D_3$ and purified as described by P. F. Brumbaugh and D. H. Haussler [*J. Biol. Chem.* **249**, 1251 (1974)].
13. P. E. Leaverton and D. V. Huntsberger, *Statistical Inference in the Biomedical Sciences* (Allyn and Bacon, Boston, 1970).
14. The circulating concentrations of 1,25-$(OH)_2D$ reported here are approximately one-third of those found in weanling rats by M. R. Hughes, P. F. Brumbaugh, M. R. Haussler, J. E. Wergedal, and D. J. Baylink [*Science* **190**, 578 (1975)] who used the same assay. This difference is probably due to the rats in the present study being older and of a different strain.
15. L. E. Schneider, L. Nowosielski, H. P. Schedl, *Endocrinology* **100**, 67 (1977).
16. M. Peterlik, K. M. Bursac, M. R. Haussler, M. R. Hughes, R. H. Wasserman, *Biochem. Biophys. Res. Commun.* **70**, 797 (1976).
17. J. E. Zerwekh, P. F. Brumbaugh, D. H. Haussler, D. J. Cork, M. R. Haussler, *Biochemistry* **13**, 4097 (1974); M. F. Holick, S. A. Holick, T. Tavela, T. Gallagher, H. K. Schnoes, H. F. DeLuca, *Science* **190**, 576 (1975).
18. L. E. Schneider, G. K. Hargis, H. P. Schedl, G. A. Williams, *Endocrinology* **95**, 711 (1974).
19. C. S. Lee, S. M. Mauer, D. M. Brown, D. E. R. Sutherland, A. F. Michael, J. S. Najarian, *J. Exp. Med.* **139**, 793 (1974); R. Weil, M. Nozawa, M. Koss, C. Weber, K. Reemtsma, R. McIntosh, *Arch. Pathol. Lab. Med.* **100**, 37 (1976).
20. K. M. Botham, J. G. Ghazarian, Y. Tanaka, H. F. DeLuca, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 661 (1975).
21. J. G. Haddad and J. Walgate, *J. Biol. Chem.* **251**, 4803 (1976).
22. ———, *J. Clin. Invest.* **58**, 1217 (1976).
23. K. R. L. Mansford and L. Opie, *Lancet* **1968-I**, 670 (1968).
24. A. F. DeNicola, O. Fridman, E. J. DelCastillo, V. G. Foglia, *Horm. Metab. Res.* **8**, 388 (1976).
25. D. V. Kimberg, R. D. Baerg, E. Gershon, R. T. Gradusius, *J. Clin. Invest.* **50**, 1309 (1971).
26. B. P. Lukert, S. W. Stanbury, E. B. Mawer, *Endocrinology* **93**, 718 (1973).
27. R. Kaplon, M. R. Haussler, C. Pak, *J. Clin. Invest.*, in press.
28. M. J. Fauvus, D. V. Kimberg, G. N. Milar, E. Gershon, *ibid.* **52**, 1328 (1973).
29. K. Wu, K. E. Schubeck, H. M. Frost, A. Villanueva, *Calcif. Tissue Res.* **6**, 204 (1970).
30. J. M. Jurist, *Phys. Med. Biol.* **15**, 427 (1970); H. W. Neuman and W. D. Arnold, *Zentralbl. Chirurgie* **96**, 1395 (1971).
31. H. W. Neuman, W. D. Arnold, W. Hein, *Zentralbl. Chirurgie* **97**, 831 (1972).
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Persistence of Hong Kong Influenza Virus Variants in Pigs

Abstract. *The A/Hong Kong/1/68 (H3N2) influenza virus which has not been isolated from man for several years, was recently isolated from pigs in Hong Kong. Influenza viruses similar to A/Victoria/3/75, which are currently circulating in man, were also isolated from pigs. Both above-mentioned viruses could be transmitted readily from pig to pig in experimental studies. The isolation of influenza viruses similar to A/Hong Kong/68 from pigs in 1976 suggests that pigs may serve as a potential reservoir for future human pandemics as well as a possible source of genetic information for recombination between human and porcine strains of influenza virus.*

Influenza viruses antigenically identical with the A/Hong Kong/1/68 (H3N2) strain from man were first isolated from slaughterhouse pigs in Taiwan in 1969 (*1*). Serological studies have shown that Hong Kong/68 influenza virus appeared in man in 1968 prior to infection of pigs with this virus (*2, 3*). The detection of antibodies to Hong Kong influenza virus in pigs in widely separated parts of the world—Germany, Hungary, Great Britain, United States, and Taiwan (*2, 3, 4*)—suggests either that this virus spreads rapidly in pigs or, more likely, that multiple transmissions from man to pigs have occurred. The Hong Kong/68 influenza virus caused no overt signs of disease in pigs, and experimental transmission to contact animals could only be demonstrated serologically (*1, 3*). Serological and virus isolation studies since 1972 indicate that the A/England/42/72 and the A/Port Chalmers/1/73 variants of Hong Kong/68 influenza virus have also been transmitted to pigs and avian species (*5, 6*). There has been no evidence to date that the Hong Kong strains have been maintained in the pig populations of the world.

We now describe (i) the characterization of A/Hong Kong/1/68–like and A/Victoria/3/75–like influenza viruses isolated from pigs in Hong Kong in 1976 and (ii) experimental transmission studies of these viruses in pigs. In man, the A/Hong Kong/1/68 variant of influenza virus has been superseded by the England, Port Chalmers, Scotland, and Victoria strains

Table 1. Identification of influenza viruses isolated from pigs in Hong Kong in May 1976 by hemagglutination inhibition. Postinfection ferret serums were provided by the Center for Disease Control Atlanta; the serums were treated with receptor-destroying enzyme and tested in HI assays with four agglutinating doses of virus as described (*8*).

Virus	HI titers with postinfection serums to:						
	Hong Kong/68 (F)*	England/72 (F)	Port Chalmers/73 (F)	Scotland/74 (F)	Victoria/75 (F)	Sw/HK/3/76 (P)*	Sw/HK/4/76 (P)
A/Hong Kong/1/68	6,000	1,600	300	200	560	160	<20
A/England/42/72	1,000	18,000	2,000	400	560	40	<20
A/Port Chalmers/1/73	150	2,000	2,000	400	300	20	20
A/Scotland/840/74	<20	300	560	2,000	150	20	<20
A/Victoria/3/75	<20	640	4,500	150	18,000	<20	640
A/swine/HK/3/76	2,500	6,400	300	150	500	160	<20
A/swine/HK/4/76	<20	300	1,000	<20	4,500	<20	320

*F = ferret serums, P = postinfection pig serums. The figures represent the reciprocals of the serum dilutions inhibiting three out of four agglutinating doses of virus.