tibody. Homotypic neutralizing antibody was found in the serum deriving from vaccinated mice; as for heterotypic antibody, experimental limitations prevented a clear-cut result indicating the significance of neutralizing antibody against Type 3 virus. It is of interest to recall in this connection that Sabin (10) found cross-neutralizing antibody to arise in man against Type 2 virus during the early stage after infection with Type 1 poliomyelitis virus. Whether the cross-protection induced with the inactivated MEF1 newborn-mouse variant would extend to include Type 1 has not as yet been investigated. Studies similar to the ones reported here would be indicated in monkeys with Type 1 virus; in these animals the oral route of infection rather than the intracerebral may possibly be used quantitatively(11). In this case more proper conditions for detecting any cross-protection between Types 1 and 2 might be available since it is known that with several other neurotropic viruses a low or even negligible degree of resistance against an intracerebral challenge proves to be considerable on challenge by peripheral routes.

Finally, in view of the greater degree of multiplication of the newborn-mouse adapted MEF1 virus in monkey testicular tissue culture(12), it is suggested that use of the adapted strain in such cultures might prove advantageous for studies similar to those reported here.

Conclusions. Experiments were carried out in mice in which an inactivated suspension of the MEF1 newborn-mouse adapted strain of Type 2 poliomyelitis virus was used as an immunizing agent. It was found that 1) the adapted strain had a far greater immunogenic potency than the standard when the animals were challenged with the homotypic strain; and 2) a significant and reproducible cross-protection was detected when the mice were challenged intraspinally with a heterotypic, Type 3 (Leon) strain.

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Statistical Analysis of Renal Clearance by the Dog.* (19746)

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A lack of adequate references in the literature to the values of normal renal function in the dog made it apparent that a statistical analysis on a large number of data obtained in this laboratory might be of general use. Therefore, a statistical analysis of "normal" renal clearance was performed on the data accumulated over a period of 6 years using 31 female dogs. The measurements of renal function included glomerular filtration (creatinine clearance)(1), p-aminohippurate (PAH) renal plasma flow and Tm(2), arginine Tm(3), and "filtration fraction" of penicillin-G clearance(4,5) compared with filtration fraction of para-aminohippurate clearance. No attempt was made to replicate

TABLE I. Mean Creatinine Clearance Values for Dogs, Expressed as ml/min/M².

Dog	Year 1943 mean	1944 mean	1945 mean	1946 mean	1947 mean	1948 mean	1949 mean	Grand mean
1	80 (51)*	83 (75)	76 (76)	91 (44)	100 (21)	—	—	83
2	121 (2)	—	—	—	—	—	—	121
3	89 (2)	—	—	—	—	—	—	89
4	67 (5)	—	—	—	—	—	—	67
5	—	83 (45)	115 (11)	—	—	72 (2)	—	88
6	82 (59)	86 (72)	105 (15)	97 (94)	101 (77)	96 (40)	74 (25)	92
7	—	84 (57)	59 (8)	—	—	—	—	81
8	—	64 (2)	102 (14)	—	—	—	—	97
9	88 (13)	—	—	—	—	—	—	88
10	—	125 (8)	—	—	—	—	—	125
11	—	—	113 (16)	—	—	—	—	113
12	—	—	100 (18)	—	—	—	—	100
13	—	—	80 (34)	94 (38)	93 (31)	75 (6)	—	88
14	71 (2)	—	91 (38)	86 (73)	86 (23)	—	—	87
15	—	—	113 (4)	—	—	—	—	113
16	—	—	89 (3)	—	—	—	—	89
17	—	—	83 (3)	107 (83)	114 (80)	—	—	110
18	—	—	87 (3)	94 (78)	92 (79)	79 (24)	—	91
19	—	—	—	—	101 (6)	59 (2)	—	90
20	—	—	—	—	170 (3)	—	—	170
21	—	—	—	—	—	98 (3)	—	98
22	—	—	—	111 (13)	113 (18)	70 (9)	—	103
23	—	—	—	—	124 (8)	—	—	124
24	—	—	—	—	—	93 (77)	92 (21)	93
25	—	—	—	—	120 (2)	116 (3)	—	118
26	—	—	—	90 (16)	91 (47)	87 (18)	81 (4)	90
27	—	—	—	99 (18)	104 (60)	142 (12)	—	108
28	—	—	—	—	110 (12)	109 (77)	—	109
29	—	—	—	—	—	104(131)	90 (14)	102
30	—	82 (10)	—	—	—	—	—	82
31	—	—	—	—	—	—	74 (12)	74
Grand mean	82	85	89	96	101	99	83	$\bar{\bar{X}} = 94$

* No. of determinations.

the experiments equally for all dogs, since some of the animals were not always available for experimentation over the entire range which is covered by this study. All values presented here are as per sq. M of surface area, calculated from the Meeh-Rubner equation:

$$SA = \left(\frac{11.2 \times W^{2/3}}{100} \right)$$ where SA = surface area, and W = wt in kg

The determination of all creatinine for measurements of glomerular filtration in this study was obtained by the method of Folin and Wu(6). Para-aminohippurate values were obtained by the method of Smith *et al.*(2). All arginine values were determined by the method of Stokes *et al.*(7). In the course of these studies the penicillin assays were those of Rammelkamp(8), Schmidt and Moyer(9), and Oliver(10).

A test for interaction between dogs and years was determined on a total of 2,045 determinations of creatinine clearances using a weighted method described by Snedecor(11). It was found by this analysis that large discrepancies existed among dogs from year to year (Table I). The standard deviation of a single determination on any given dog for any one occasion was 9 cc/min./sq. M surface area. The 95% confidence limits were 18 cc/min./sq. M surface area. Ninety-five per cent of all observations in this study may be expected to fall within the limits 94 ± 36 cc/min./sq. M surface area.

The analysis of 290 determinations of p-aminohippurate renal plasma flow showed that the mean values differed from dog to dog and from one occasion to another (Table II). The standard deviation of a single determination

TABLE II. Mean p-aminohippurate Renal Plasma Flow Values for Dogs, Expressed as ml/min/M².

Dog No.	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{\bar{X}}$
Mean value	160	184	244	283	211	279	226	324	199	285	233	283	238
No. of determinations	20	47	103	2	3	6	3	17	9	2	32	46	290

TABLE III. Analysis of Variance.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F ratio*
Between dogs	6	54.9043	9.1507	2.83
Within dogs	13	41.9710	3.2285	
Total	19	96.8753		

* F ratio = Ratio of mean² for between dogs to the mean² within dogs.

on any given dog for any one occasion was 32 cc/min./sq. M surface area. The 95% confidence limits were 63 cc/min./sq. M surface area. Ninety-five per cent of all observations in this study may be expected to fall within the limits 238 ± 133 cc/min./sq. M surface area.

It was not possible to do an extensive analysis of the p-aminohippurate Tm values, since only 20 values spread over 7 dogs were available. In this analysis the variation between dogs was not significant and could be attributed to experimental error (Table III). The standard deviation of a single determination on any given dog for any one occasion was 1.8 mg/min./sq. M surface area. The 95% confidence limits were 3.9 mg/min./sq. M surface area. Ninety-five per cent of all observations in this study may be expected to fall within the limits 16.5 ± 4.7 mg/min./sq. M surface area.

The analysis of arginine Tm was made on 27 values from dogs variously employed over the years 1946, 1947 and 1949. The statistical method used was an adaptation of a procedure of Ganguli(12). The mean arginine Tm value for 1949 was significantly lower than that for 1946 and 1947. The difference between dogs was not significant and may be a reflection of differences between years. Since the difference between years was significant, the estimation of a general mean arginine Tm value is not too meaningful. We report it here for completeness (Table IV). The standard deviation of a single determination for any given dog for any one occasion was 1.7 mg/min./sq. M surface area. The 95% confidence limits were 3.3 mg/min./sq. M surface area. Ninety-five per cent of all observations in this study may be expected to fall within the limits 12.9 ± 7.6 mg/min./sq. M surface area.

There was a total of 919 values on 10 dogs available for study of the "filtration fractions" of penicillin clearances as compared to the filtration fractions of p-aminohippurate clearances. It was evident from a preliminary analysis on all of the values that the variations among the filtration fractions of penicillin and the filtration fractions of p-aminohippurate values from dog to dog and from one occasion to another were greater than those observed in the same dog on the same occasion. Therefore, replicate determinations for every occasion were averaged and these values (63 for filtration fraction of p-aminohippurate and 207 for filtration fraction of penicillin) were used in the actual analyses. Because of the great disproportionality of the data, a weighted analysis of variance was done according to the method of Snedecor(11) (Table V). The weighted mean value for the filtration fraction of p-aminohippurate was .40. The difference between the weighted value of penicillin and the weighted value of p-aminohippurate was .05. In general, there is a consistent trend for the filtration fraction of p-aminohippurate values to be slightly higher than the filtration fraction of penicillin values. This is highly significant statistically ($P < 0.01$ and close to $P = 0.001$). Actually, in spite of the significant statistical result, we would require more data in the filtration fraction of the p-aminohippurate group of figures to be sure that differences noted are real and not a reflection of disproportion.

TABLE IV. Mean Arginine Tm Values for Dogs, Expressed as mg/min/M².

Dog No.	Date of exp.	Arginine clearance values: Mean value for date	Mean value for year	Mean value for dog
370	9–17–46	15.26	15.55	17.27
	9–19–46	15.84		
	10–30–47	20.71	20.71	
365	9–17–46	13	12.07	12.23
	9–19–46	11.14		
	10– 2–47	16.08	14.27	
	11–13–47	13.07		
	6–23–49	7.44	7.44	
291	6–13–49	7.26	7.97	7.97
	6–23–49	8.67		
84	7–12–46	15.02	15.02	12.94
	6–10–49	8.79	8.79	
General mean				12.88

TABLE V. Mean Values and Number of Occasions for the Determination of p-aminohippurate and Penicillin—G "Filtration Fractions" for Each Dog.

Dog*	A F.F. (PAH)	B F.F. penicillin—G	Difference (A)–(B)
1	.46 (8)	.34 (31)	.12
2	.41(24)	.39 (16)	.02
3	.36 (1)	.30 (5)	.06
4	.41 (1)	.42 (42)	–.01
5	.43 (2)	.35 (50)	.08
6	.31 (3)	.32 (55)	–.01
7	.44 (3)	.32 (60)	.12
8	.42 (1)	.24 (1)	.18
9	.37 (9)	.35 (5)	.02
10	.37(11)	.36 (5)	.01
Weighted mean	.40(63)	.35(270)	.05

* Dog numbers are tabular and are not necessarily the same for each animal from table to table.

Comment. Since so few data, except representative experiments, appear in the literature we have felt it justifiable to present this analysis of "normal" values for canine renal functions despite its limitations. The limitations imposed on this study derive principally from the fact that the data used were the control values from experiments designed for other purposes. Perhaps the statistical analysis has suffered from the limitation of unequal numbers of values and what a statistician would consider an unorderly sequence of accumulation.

The analysis of creatinine glomerular filtration (GF) rate is the most reliable because of the large number (2,045) of accurate determinations on the greatest population. Even so, there were insufficient data over a long enough span of the animals' lives to indicate a trend to any change in GF within a 3- to 7-year span.

It was surprising that the filtration fraction (FF) as represented by GF/RPF_{PAH} determined simultaneously should be so high (0.40), as compared to an FF of 0.19(13) for man. Indeed, we customarily found the clearance of penicillin-G at concentrations of 2 units/ml of plasma to exceed or equal the apparent RPF for PAH measured at different times in the same animals. It may be that the values for RPF were influenced by our customary oral administration of PAH. Poor gastrointestinal absorption was made use of to maintain a quite constant PAH plasma concentration of the order of 1.0 mg/100 ml or less following the administration of the drug *per os*. However, recent reports have suggested that in man RPF values were lower when PAH was administered orally than when it was injected parenterally(14).

It is tenable in the absence of suitable evidence that the small portion of PAH absorbed is either partially hydrolyzed to p-aminobenzoic acid and glycine or conjugated as the

glucuronide within the intestine on the portal system. Either of the latter p-amino compounds would be incorporated into the analysis for PAH and would decrease the apparent clearance of that compound. Checks of various lots of PAH from our source have yielded no evidence that the material administered was contaminated with p-aminobenzoic acid.

In spite of the greater inherent error in the microbiological assay, the "filtration fraction" for simultaneously determined creatinine and penicillin-G clearances is probably the more reliable figure, although that agent is not commonly employed for the measurement of renal plasma flow. Penicillin-G does have the advantage, though, that seemingly large variations in microbiologically active plasma concentrations have no effect on its clearance.

While the number of Tm_{PAH} determinations were few they indicated a satisfactory order of reproducibility of the values for any animal within an experiment, from day to day and for a population of apparently normal dogs.

The Tm values for arginine were found to be as reproducible within repeated tests in a given experiment or from day to day as obtained for PAH. However, the variation of the values from year to year was evident for each dog where comparisons could be made. Although this fluctuation is strictly unaccountable, it may be thought to be due to subtle changes in the microbiological assay of the compound from time to time.

Lastly, it should be pointed out that these data all represent values for the female. Indeed, there seem to be insufficient information in the literature to judge whether systematic sex differences in these functions obtain in the dog.

Summary. From data accumulated over a 6-year period on 31 trained, unanesthetized female dogs the following mean values for several renal functions were calculated: glomerular filtration rate (creatinine clearance) = 94 ml/min./sq. M surface area; renal plasma flow (p-aminohippurate clearance following its oral administration) = 238 ml/min./sq. M; p-aminohippurate Tm = 16.5 mg/min./sq. M; arginine Tm = 12.9 mg/min./sq. M; simultaneous (creatinine) GF/p-aminohippurate RPF filtration fraction = 0.40; and GF/penicillin RPF "filtration fraction" = 0.35. A statistical analysis of the variability of the results was presented and the significance of the data was discussed.

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