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ABSTRACT

Simple environmental changes, including a whole, fresh diet, delayed the onset of the usual diseases of lungs and kidneys in laboratory rats. 'Spontaneous' nephrosis appears to develop from interactions involving inherent immune status, nurture, and environmental 'triggers', particularly inhalants. These methods could be adapted to toxicological investigations and the results suggest the need for new approaches in statistical and interpretative analyses.

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AGE DEPENDENCE; DIET; DISEASES; INHALATION; KIDNEYS; LATENCY PERIOD; LUNGS; PROTEINS; STATISTICAL MODELS; RATS; SEX RATIO; SYNERGISM; URINE

CONTENTS

1. INTRODUCTION

The need is acknowledged for development of methods in experimental toxicology using whole animals as subjects (1). Differences will occur in the outside influences upon and internal reactions of individuals and whole populations under study, making interpretation an onerous task. In this paper we describe how comparable populations were kept under conditions of well defined diversity. From simple, non-destructively measured parameters, processed by computer into diagnostic patterns, we sought indications of any correspondingly different reactions and an insight into basic toxicological mechanisms.

In earlier work we remarked that the one feature common to reported experiments, which ameliorated nephrosis in laboratory rats, was a modified routine for animal keeping (22). Since the experimental methods varied, sometimes in contradictory manner, we thought it possible that any advantage might lie in the changed experimental environment, especially where feeding practice was altered. This, together with our identification of food-cube particles in peribronchial lesions of the murine chronic respiratory disease (CRD) which developed in our controls, suggested common aetiological bases for CRD and the 'spontaneous' murine nephrosis (3) in cube-fed colonies of rats.

We have reported the elimination of the stigmata of CRD from lungs of a group of rats (M/F below) which we bred and kept in an atmosphere free from food particles and mixed litter dusts (24). There is increasing evidence that CRD consists of hyperplasia of lymphoid foci in the lung clearance pathways, with consequent partial obstruction of the bronchi and superadded retrograde infection of the lower respiratory tract by mycoplasmas (PPLOs) (19). Increased susceptibility to the establishment of lung lesions following intranasal inocula of *mycoplasma pulmonis* was noted in rats specially raised and confined with some crowding in plastic-film isolators;

this was attributed to unspecified environmental effects (16).

Cameron (8) reviewed clinical and experimental evidence for involvement of systemic effects, infectious and immune, in the development of human renal disease and urged the need to search in closed environments for non-immunological triggers of immune-mediated processes. It seems that blood circulating through infected lungs could be expected to disseminate degradation and specific-immune products (23), especially to the kidneys.

2. METHODS

The 'clean' colony was established in stainless steel cages of standard size with mesh floors, placed on three-tiered mobile racks in part of the medical suite which was air-conditioned at 21° to 23° Celsius. Absorbent paper underlays were changed daily. We took 2 pairs of weanling albino rats as breeding stock from a strain maintained in the established animal house and known to develop the usual laboratory diseases of lungs and kidneys. Strawlike paper offcuts were supplied for the breeding nests because we had observed that nursing does were continually digging their young from the sawdust nests. (Lane-Petter recently questioned the use of sawdust (15).) Gravid females, with rare exceptions, built fully enclosed nests of straw within minutes of it being supplied and the young emerged only as the time of weaning approached. Those that failed to build nests proved to be inadequate mothers, often losing an entire litter.

To minimise the generation of food dusts, we devised a protein-rich diet of fresh foods, served 'whole' (Table 1.) All food was of domestic quality. Vegetables were rinsed as in kitchen practice and no sterilisation was applied other than any necessary cooking. Food was placed in the cages in the late afternoon and the debris was removed with the paper underlay next morning. A slight excess over demand at all stages ensured rates of growth equal to that of cube-fed rats (Figure 1; Tables 2 and 3). Human contacts were encouraged, interested staff freely visited the colony and

contract cleaners serviced the area without supervision.

Over 50 males and as many females were retained after weaning in the 'clean' colony as the main experimental group (M/F). About half as many males and females were transferred when weaned to a separate cubicle of the established animal house to form an environmental control group (A/F). There they were given the fresh diet and caged in exactly the same way as the M/F group. However the cubicle of the animal house was air-conditioned in common with all other cubicles by a partly recirculating air-conditioner, exposing the A/F group in some degree to the mixed litter dusts of a variety of experimental animals. A small dietary reference group (A/C), bred also in the 'clean' colony, was placed in another of these cubicles and fed *ad libitum* on commercially prepared rat cubes. The numbers in this group, 6 of each sex, were small but seemed adequate because we wished only for some measure against published results; even where such reports referred to large colonies (3), samples taken were small, random, and tested much less frequently throughout a lifespan than in our method.

Twelve male rats of the M/F group were housed in one large cage, with floor space 33 x 18 inches and height 27 inches to allow free climbing. We sought maximum weight gains in this group on the fresh diet and they filled the upper quartile of body weights for the M/F males (Figure 1a; Table 2). The 4 rats from the B_2 litter were albinos; the 8 from the MHX_2 litter were from a Manchester Hooded/albino-cross doe backcrossed to an albino sire. (The crossed litter and 2 subsequent backcross litters disproved in our experience the assertion that such progeny are more susceptible to nephrosis (12.).

3. RESULTS

3.1 Parameters and Patterns

Rats were weighed during the first week of each calendar month, then placed in individual metabolism cages supplied only with water for collection

of a 24-hour urine sample. Urinary protein concentrations were determined by the sulpho-salicylic acid method, using a grey-wedge photometer (14). Body weight, urine volume, protein concentration, and date of estimation were entered in the computer program, each litter being identified with its birthdate. From the raw data it was possible to plot either

- all individual points against age in days; or
- grouped analyses for chosen age intervals.

No conclusions can be drawn about lifespans since we sacrificed rats for the respiratory studies which had been our prime purpose in establishing this colony. Any rat showing first signs of morbidity which might result in secondary changes in lungs at postmortem was sacrificed. This shortening of lifespans was exaggerated since the latest entry against any rat was that recorded when it was last judged fit to spend 24 hours in the isolation of the metabolism cage.

Once initiated,the nephrotic process resulted in proteinuria which increased in individual rats by near parallel, steadily progressive courses as exemplified by the 12 rats of the M/F male subgroup (Figure 2). Each such course was characterised by :

- a latent period, of length peculiar to the individual;
- increasingly steep progression, with short reversals; and
- some flattening off in the fully developed stage.

Distinctive group patterns resulted from analyses for each of the subgroups under observation (Figure 3; Tables 2 and 3.). Passing from the A/C subgroups, exposed to both food and mixed litter dusts, to the A/F subgroups, exposed to mixed litter dusts only, and to the M/F subgroups, which encountered neither food nor mixed litter dusts, we find that there was a marked shift to the right (Figure 3.), corresponding with a delay in age before the more favoured groups reached corresponding levels of urinary protein output. Females in each situation were significantly

slower than their male counterparts to develop nephrosis and retained that sex-linked advantage in addition to any changes wrought by environmental and dietary modifications. Histological evidence for delays of similar degree in the incidence of age-related diseases in females, vis-a-vis males, was reported by Berg and associates (4).

The urinary protein output of the B_2 litter males proved them the most susceptible of the M/F males to nephrosis (Figure 2). Exclusion of the data for the B_2 females (8 in number) from their subgroup analyses markedly reduced those totals - a dotted line in Figure 3b shows the reduced level of the upper extreme after their exclusion. Although the B_2 males developed nephrosis well in advance of their MHX_2 cagemates, despite identical conditions and chances for intercurrent infections, they nevertheless lagged behind the A/C males (Figure 4).

3.2 Age-specific Prevalence as a Measure of Latency

Although our criteria for sacrifice of rats, which led to an accelerated rate of attrition in the populations under study, were unrelated to the course of nephrosis in the selected individuals, there is the possibility that the shifts in group totals of proteinuria had been influenced by those policies. To resolve this uncertainty, we scored each animal in the study at its age of transition from health to disease and calculated P, the age-specific prevalence of nephrosis. From examination of computer printouts we found that the 24-hour urinary protein output showed minor, reversible fluctuations in individuals within the period of latency until a certain threshold was exceeded. At this point the progressive course of nephrosis in that individual became irrevocable. In our series this threshold was at 15 mg/24 hours in males and 10 mg/24 hours in females. The male/female body weight ratio was also 3:2, which indicates that the upper limit of urinary protein excretion in health is presumably a function of body mass. All

animals were scored positive at the actual age of transition or at age of death + 30 days, so that attrition of the populations would not bias these measurements in favour of our environmental modifications.

The data for P were fitted to the general expression for age-specific prevalence (5) :

$$(P) = S.(1 - e^{-k.(t - \delta)})^n,$$

where S = 1, representing the fraction of the population at risk;

t = age in days;

δ = length of the period of latency in days; and

n = power at which best fit of data was obtained.

Table 4 lists the computed results drawn from solution of the variables (k and δ) at all powers of n from 1 to 7; in all groups the lowest standard deviations of the means were obtained with n = 5. The resulting curves showed progressive shifts to the right of the same order and magnitude as for the group totals of proteinuria and confirmed the sex-linked advantage of the females (Figures 3, 4 and 5). The increased susceptibility of the B_2 litter was also apparent for both sexes but obviously at a lower level than in the A/C cube-fed rats. Hence, although the A/C sample was very small, hypersusceptibility alone would not suffice to account for their shortened latency which appears particularly significant in the females.

4. DISCUSSION

We turned to computer analysis at an early stage when progressive hand plotting of all individual points against age-in-days revealed that the urinary protein outputs became heavily skewed, especially in the healthier groups, whereas body weights were normally distributed at all ages. It is now recognised more widely that the use of measures of central tendency, such as the mean and standard deviation, is inappropriate when

dealing with other than normally distributed functions (21), and could be more misleading than was once thought(17). Curves drawn through the means and standard deviations taken from Berg's calculations (3) would suggest a relatively normal distribution (Figure 3).

Early recognition of skewness in the protein outputs was aided by the prolonged complete latency, up to 100 days of age throughout the period of rapid growth, shown in all of our groups including the A/C males. This contrasts with published reports and emphasises the case for environmental and dietary modification in experimental rat colonies (3, 10, 18). Age of 100 days and body weight of 200 grams are common criteria for selection of rats for experiments. The extended latency of the A/C male subgroup might be a result of our use of paper straw in the breeding nests since they, too, were raised till weaning under 'clean' colony conditions.

The persistence of endemic laboratory diseases has presented great difficulties in the interpretation of experiments in which whole animals have been used to examine problems in toxicology and pathology. These difficulties persist despite elaborate preventive measures (16). Since the tissues, and the kidney in particular, have only a limited range of response to whatever injurious stimuli are applied, murine nephrosis might be regarded, not as an endemic disease, but as a summation of immune-mediated 'stresses' during a life spent in a given environment (6). This would be consistent with failure to 'prevent' the disease. If environmental as well as infective agents cause renal damage in rats following inhalation, confusion in interpretation must follow when a toxin is superadded experimentally by the same route. Application to human working conditions of conclusions drawn from toxicological studies performed with infectively diseased animals (20) kept in unspecified environmental

conditions needs careful review.

The 'lag-phase', which occurred persistently between exposure of animals to inhaled uranium compounds and the first signs of renal damage, led Dounce (11) to suggest that the kidney was the most likely target for injury subsequent to widespread damage to 'enzyme proteins' by uranium circulating at low concentrations. Cameron made more specific suggestions as to the systemic injuries likely to mediate eventual renal damage, and thought both surface damage and coagulation might emerge as factors of equal importance to soluble complexes (6, 7). The particular sensitivity of the rat kidney to inhalants might rest on the high fibrinolytic activity and low content of thromboplastin in the rat lung as described by Astrup and associates (2). Thus, instead of lung injury leading to fibrotic repair in the rat as in other species, more breakdown products would be passed through the circulation to the kidney (23). Clarkson et al. found that the extent of fibrin(ogen) degradation product (FDP) excretion in human diseases was closely related to the degree of proteinuria (9). Ward and Preston investigated the renal failure which frequently complicates the course of myelomatosis, and decided that deposition of fibrin in renal capillaries was not the consequence of preexisting renal disease but had some aetiological significance (25). Such opinion is supported by a considerable and growing body of clinical and experimental observations (26).

5. CONCLUSIONS

The rat maintained under controlled, closed environmental conditions would appear to be a sensitive indicator and significant model of the effects of hereditary, infectious and environmental factors in the aetiology of renal disease (13, 19). Given a colony well characterised by ongoing computer analysis in such an environment, the calculated introduction of an additional toxic agent might be expected to produce measurable deviations of patterns similar to those produced by variation of factors within our control.

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Reference	9	(1971)
"	22	(1967), 215:
"	25	(1974)

TABLE 1

FRESH FOOD REQUIREMENTS

BASIC FOOD REQUIREMENTS for 4 rats daily.

VEGETABLES

Greens:
lettuce, half young heart twice weekly, or carrot tops, small bunch when available.

1 other vegetable in season on other days, from:

pumpkin	1.0 kg
melon	1.5 kg
cucumber	0.5 kg or
apples	1 medium size

PROTEIN FOODS

calves liver (boiled)	200 g twice weekly, and
cheese	120 g on other days
bread (protein enriched)	100 g daily
BUTTER	10 g spread on bread twice weekly
WATER	in 1 pint dropper bottle changed daily and sterilised

Cheese, liver, and bread are cut into individual cubes to prevent fighting. Vegetables are left in bulky pieces to conserve freshness.

ESTIMATED DIETARY CONSTITUENTS for each rat daily:

Wet weight 300 g; dry weight 55 g; protein 25%; carbohydrate 55%; fats 11%; kilocalories 200.

TABLE 2

URINARY PROTEIN OUTPUT AND BODY WEIGHT DATA OF MALE SUBGROUPS

FOR SELECTED 30-DAY COLLECTION PERIODS

X indicates, by its position across the head of each column, the mid-point of the age group; the number of survivors in each group appears immediately underneath it. 'x' marks the upper and lower extremes; 'q' the quartiles; sk. = skewness; kurt. = kurtosis.

0 100 200 300 400 500 600 700 800								
age (days)	X	X	X	X	X	X	X	X
rats	52	49	48	44	35	30	16	14
M/F-males								
URINARY PROTEINS - mg/24hrs.								
x		17.60	29.12	122.24	205.20	347.60	300.80	479.36
q		8.41	12.75	19.62	47.52	88.48	144.00	252.00
Mean (s.d.)		7.24(4.42)	9.95(5.88)	20.23(24.83)	43.17(55.01)	80.66(89.70)	107.76(90.27)	175.52(143.67)
median		7.17	8.67	11.06	18.88	48.56	92.96	122.88
q		5.70	5.55	6.21	10.92	23.60	33.00	66.88
x		3.36	2.85	2.38	3.04	5.58	9.80	22.56
sk. (kurt.)		1.449(4.620)	1.511(2.129)	2.398(5.703)	1.805(1.959)	1.741(2.015)	0.707(-1.627)	0.797(-0.508)
M/F-males								
BODY WEIGHTS - g.								
x	284	531	532	682	726	764	779	760
q	246	404	448	507	577	603	643	610
Mean (s.d.)	216(35.34)	385(46.45)	424(45.77)	467(77.76)	512(87.99)	517(97.99)	575(82.16)	549(103.07)
median	210	388	421	460	506	516	536	547
q	186	349	386	417	464	466	527	520
x	159	304	348	283	320	310	478	331
sk. (kurt.)	0.251(-1.081)	0.962(1.629)	0.463(-0.319)	0.419(0.353)	0.046(-0.106)	0.252(0.032)	0.996(0.296)	-0.113(0.840)

0 100 200 300 400 500 600 700 800								
age (days)	X	X	X	X	X	X	X	X
rats	26	26	23	19	18	17	11	5
A/F-males								
URINARY PROTEINS - mg/24hrs.								
x		11.59	31.92	211.68	235.20	385.20	566.72	212.80
q		8.16	14.80	32.67	113.56	166.08	313.60	161.52
Mean (s.d.)		7.14(2.75)	11.65(8.73)	30.88(44.42)	78.54(64.41)	140.65(90.76)	237.14(157.11)	139.02(53.02)
median		6.16	10.54	13.52	63.36	127.84	220.96	134.64
q		5.25	7.38	8.25	23.10	101.76	143.36	116.80
x		3.96	5.20	4.10	0.0	14.00	7.10	69.74
sk. (kurt.)		0.600(-1.076)	1.755(3.436)	2.940(8.366)	0.744(-0.544)	1.258(1.920)	0.602(-0.251)	0.129(-0.898)
A/F-males								
BODY WEIGHTS - g.								
Mean (s.d.)	184(16.01)	341(28.10)	400(29.66)	422(36.75)	475(60.27)	504(66.30)	457(76.01)	470(96.89)
median	186	344	403	426	493	521	447	451
sk. (kurt.)	-0.420(0.544)	-0.418(-0.504)	-0.224(-0.291)	-0.393(-0.458)	-1.192(1.269)	-0.534(-0.528)	0.441(-0.660)	-0.149(-1.203)

0 100 200 300 400 500 600 700 800								
age (days)	X	X	X	X	X	X	X	X
rats	6	6	6	5	5	4	4	
A/C-males								
URINARY PROTEINS - mg/24hrs.								
x	10.88	91.80	227.36	225.60	322.32	649.60	559.36	
q	10.32	57.96	190.08	220.32	264.96	331.76	554.40	
Mean (s.d.)	9.65(1.09)	44.41(38.09)	110.37(86.32)	146.60(92.40)	213.46(102.40)	345.66(213.82)	410.44(218.23)	
median	10.28	38.88	96.34	162.16	221.76	283.96	493.68	
q	10.25	19.80	37.60	104.00	178.56	236.16	432.96	
x	8.36	8.10	14.52	36.42	88.00	165.12	95.04	
sk. (kurt.)	-0.919(-0.803)	0.321(-1.484)	0.240(-1.533)	-0.253(-1.666)	-0.211(-1.396)	0.822(-0.929)	-0.928(-0.880)	
A/C-males								
BODY WEIGHTS - g.								
Mean (s.d.)	331(23.24)	442(49.50)	508(53.82)	443(54.56)	475(25.68)	446(54.21)	426(89.58)	
median	332	425	496	451	478	442	392	
sk. (kurt.)	-0.029(-1.951)	0.781(-1.108)	0.552(-1.299)	-0.664(-0.652)	0.026(-1.969)	0.232(-1.308)	0.972(-0.834)	

TABLE 3

URINARY PROTEIN OUTPUT AND BODY WEIGHT DATA OF FEMALE SUBGROUPS

TO CORRESPOND WITH TABLE 2

0 100 200 300 400 500 600 700 800								
age (days)	X	X	X	X	X	X	X	X
rats	58	52	52	52	48	37	28	21
N/F-females								
URINARY PROTEINS - mg/24hrs.								
x		3.74	50.96	122.88	250.56	322.56	137.60	112.00
q		1.98	2.88	4.06	7.56	24.00	18.56	44.16
Mean (s.d.)		1.29(1.77)	2.90(5.83)	6.45(15.35)	14.28(34.45)	25.18(48.74)	18.48(30.61)	28.09(30.60)
median		1.44	1.92	2.70	5.04	6.76	7.53	14.42
q		0.0	0.99	1.80	2.52	4.08	3.04	3.51
x		0.0	0.0	0.0	0.0	1.26	1.44	0.99
sk. (kurt.)		0.264(-0.819)	6.759(49.383)	5.719(36.169)	4.896(26.688)	4.644(20.492)	2.766(6.787)	1.210(0.609)
N/F-females								
BODY WEIGHTS - g								
x	288	378	445	457	579	418	437	447
q	224	268	293	309	345	341	350	350
Mean (s.d.)	207(26.14)	254(34.63)	279(42.71)	291(58.09)	325(73.26)	311(43.24)	320(49.31)	320(53.59)
median	204	249	272	286	305	305	314	328
q	187	236	248	249	280	285	291	286
x	172	194	215	198	240	244	223	225
sk. (kurt.)	0.969(0.704)	1.404(3.033)	1.649(3.266)	1.136(1.490)	1.679(2.763)	0.509(-0.246)	0.466(0.033)	0.269(-0.059)

0 100 200 300 400 500 600 700 800								
age (days)	X	X	X	X	X	X	X	X
rats	21	20	20	18	17	16	12	9
A/F-females								
URINARY PROTEINS - mg/24hrs.								
x		4.42	10.50	38.00	140.80	282.24	292.80	126.56
q		3.24	4.00	6.60	40.16	87.12	87.36	124.64
Mean (s.d.)		2.72(0.88)	3.77(2.03)	7.05(7.89)	28.58(37.34)	64.52(74.25)	68.22(70.17)	88.36(42.14)
median		2.64	3.66	5.05	14.10	36.24	51.54	106.56
q		1.98	2.70	3.08	4.00	10.36	21.12	46.20
x		1.68	0.88	1.26	1.00	2.64	4.65	15.39
sk. (kurt.)		0.405(-0.947)	1.582(3.364)	2.976(8.602)	1.718(2.045)	1.383(1.072)	1.971(3.681)	-0.829(-0.798)
A/F-females								
BODY WEIGHTS - g								
Mean (s.d.)	147(10.23)	261(19.98)	261(19.98)	291(24.72)	308(25.73)	310(48.48)	329(53.25)	324(65.01)
median	148	260	260	296	316	309	317	305
sk. (kurt.)	-0.555(0.358)	-0.015(-1.171)	-0.166(-1.463)	-0.242(-0.443)	-0.542(-0.315)	0.274(-0.818)	0.703(-0.484)	0.473(-0.908)

0 100 200 300 400 500 600 700 800								
age (days)			X	X	X	X	X	X
rats			6	6	6	6	5	3
A/C-females								
URINARY PROTEINS - mg/24hrs.								
x			10.35	39.56	65.28	192.78	203.84	248.00
q			8.16	20.00	36.40	148.74	184.80	
Mean (s.d.)			5.40(3.09)	15.22(13.34)	27.43(22.35)	91.03(58.09)	115.73(72.94)	107.80(122.97)
median			3.93	10.75	23.59	56.16	73.20	57.20
q			3.15	5.52	8.32	38.76	72.00	
x			2.88	4.76	7.40	18.72	44.80	18.19
sk. (kurt.)			0.802(-1.078)	1.092(-0.147)	0.738(-0.704)	0.441(-1.521)	0.357(-1.728)	0.627(-1.500)
A/C-females								
BODY WEIGHTS - g								
Mean (s.d.)			289(14.07)	317(16.39)	300(19.97)	306(22.52)	314(32.20)	271(52.00)
median			294	322	308	315	297	271
sk. (kurt.)			-0.767(-0.933)	-0.567(-1.188)	-0.557(-1.494)	-0.622(-1.101)	1.134(-0.353)	0.00 (-1.500)

TABLE 4

VALUES CALCULATED FOR 'k' AND 'δ'

Sub-group	'k'	s.d.	'δ'	$\frac{\Delta P}{\Delta t}$ (20–80%) = 32 x 'k'
M/F male	.008	.0002	106.4	0.26% days^{-1} = 32 x .008
A/F male	.011	.0004	116.3	0.35 = 32 x .011
A/C male	.042	.009	98.0	1.3 = 31 x .042
M/F female	.006	.0001	223.4	0.20 = 33 x .006
A/F female	.008	.0007	194.5	0.25 = 32 x .008
A/C female	.011	.0014	157.7	0.35 = 32 x .011

These values for 'k' and 'δ' were obtained when the data for P was fitted at 'n = 5'. $\frac{\Delta P}{\Delta t}$, the net slope of the nearly straight parts of the resultant curves between P = 20% and P = 80%, was found by observation to be equal to 32 times 'k' in each of the cases.

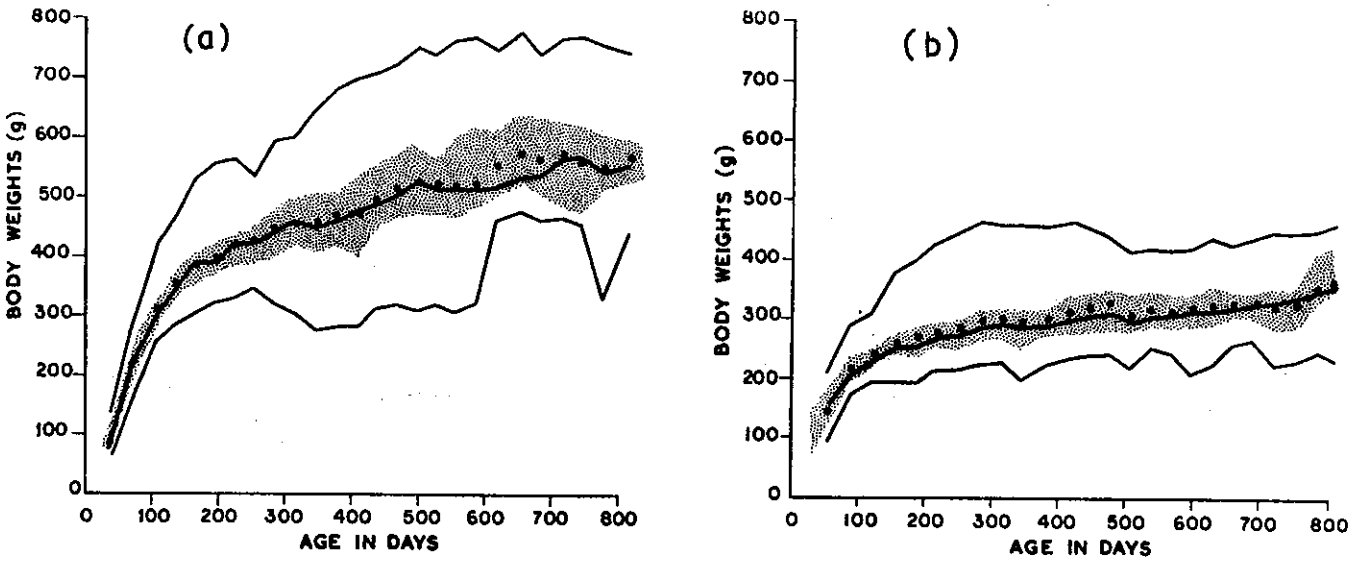

FIGURE 1. GROWTH CURVES FOR M/F MALES (a) AND M/F FEMALES (b) PRESENTING EXTREMES (FINE LINES), INTERQUARTILE RANGE (SHADED), MEDIANS (HEAVY LINE), AND MEANS (DOTTED)

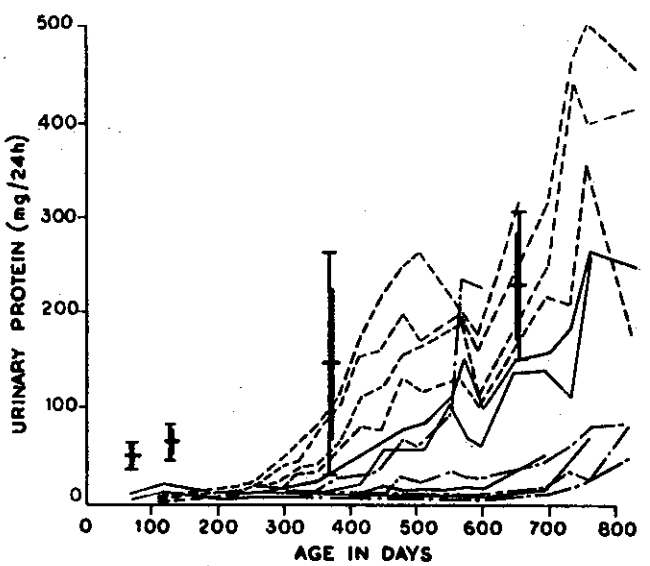

FIGURE 2. DASHED LINES INDICATE THE INDIVIDUAL CURVES FOR URINARY PROTEIN OUTPUT OF FOUR B_2 MALES, OTHER LINES FOR MEMBERS OF MHX_2 MALES

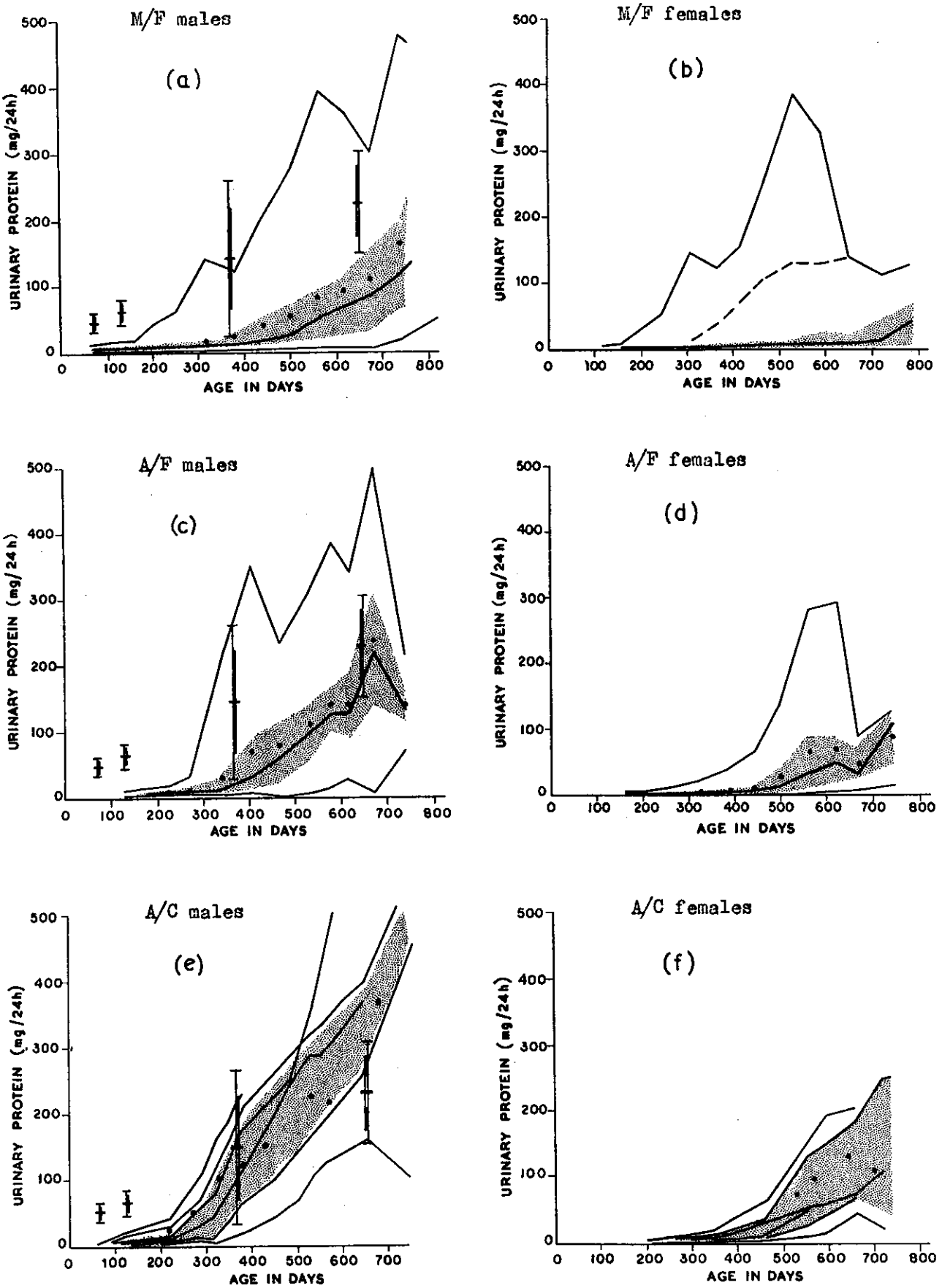

FIGURE 3. 24-HOUR URINARY PROTEIN OUTPUTS OF MALE AND FEMALE SUBGROUPS ANALYSED TO SHOW EXTREMES, INTERQUARTILE RANGE , MEDIAN, AND MEAN (DOTS). BARS SHOW MEAN $\pm$3 s.d. FROM AN S.P.F. COLONY[3] FOR COMPARISON IN MALE GROUPS

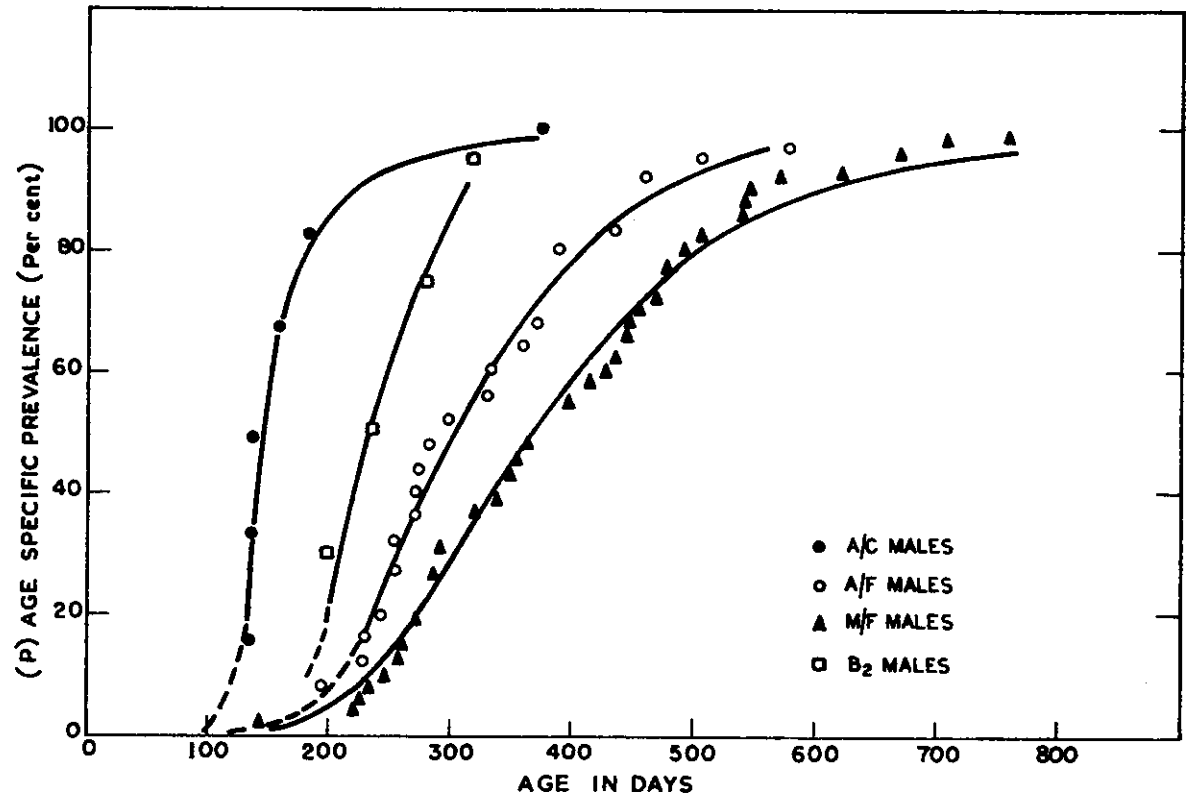

FIGURE 4. CALCULATED CURVES FOR AGE-SPECIFIC PREVALENCE SUPERIMPOSED ON THE INDIVIDUAL DATA POINTS OF MALE SUBGROUPS

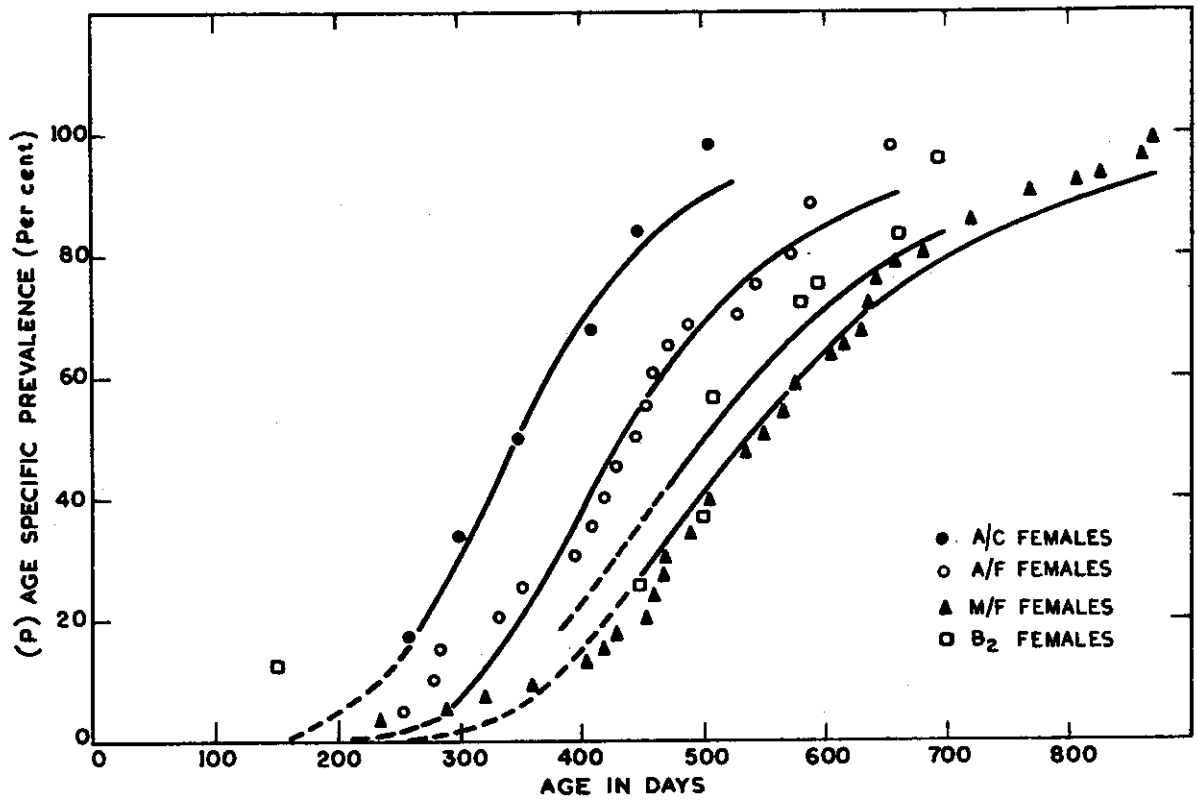

FIGURE 5. CALCULATED CURVES FOR AGE-SPECIFIC PREVALENCE SUPERIMPOSED ON THE INDIVIDUAL DATA POINTS OF FEMALE SUBGROUPS