Table 1 Susceptibility to Rifampin of Two Strains of Chlamydiae and of Their Mutants

Chlamydial strain	Inoculum ELD_{50} ml^{-1}	No drug Deaths/total	No drug ADD‡	Rifampin (20 μg ml^{-1}) Deaths/total	Rifampin (20 μg ml^{-1}) ADD‡	Rifampin (200 μg ml^{-1}) Deaths/total	Rifampin (200 μg ml^{-1}) ADD‡
TE-55 original	3,000	24/24	6.2	13/27	10.4	4/17	13.0
TE-55 resistant*	4,000	27/27	6.0	25/25	6.1	28/28	7.4
AP-2 original	50,000	29/29	6.4	1/29	12.8	0/31	13.0
AP-2 resistant †	20,000	31/31	6.8	31/31	6.8	28/28	7.8

* After five passages in eggs in presence of rifampin 5 to 200 μg ml^{-1} (2.5 to 100 μg per egg).
† After seven passages in eggs in presence of rifampin 5 to 200 μg ml^{-1} (2.5 to 100 μg per egg).
‡ ADD, average day of death of embryonated eggs.

inocula grown in the presence of increasing concentrations of the drug. Nabli[10] had reported complete inhibition of chlamydial agents in eggs by concentrations of rifampin similar to those used by us. However, Nabli's original inocula were perhaps too small (10^3 infective particles per egg) to permit rapid selection of mutants, and repeated passage of chlamydiae in eggs in the presence of rifampin had not been attempted. For one of the strains used by us (TE-55), Becker had suggested that rifampin was lethal rather than inhibitory, and that the appearance of rifampin-resistant mutants might be "extremely rare"[6]. Our results suggest that, on the contrary, chlamydiae resistant to high levels of rifampin emerge quite promptly. Thus, the selection of rifampin-resistant mutants in chlamydial populations is not fundamentally different from that in populations of gram negative bacteria[1,2]. Recently, Tribby *et al.*[11] also found that the effect of rifampin on chlamydial multiplication was inhibitory and reversible, rather than lethal as proposed earlier[5]. In view of these findings, any large scale or long term therapy of a chronic infection like trachoma with rifampin as the sole drug appears undesirable.

Limited clinical trials (C. R. Dawson, personal communication) of rifampin in trachoma have shown no advantage over tetracyclines used in the same trials. These experiences illustrate that the extrapolation of limited results, obtained in a single model system, to the clinical application of drugs in human disease may be unwarranted.

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1 Kunin, C., Brandt, D., and Wood, H., *J. Infect. Dis.*, **119**, 132 (1969).
2 Eichoff, T. C., *J. Infect. Dis.*, **123**, 414 (1971).
3 Moulder, J. W., *Perspect. Biol. Med.*, **14**, 486 (1971).
4 Dawson, C. R., Ostler, H. B., Hanna, L., Hoshiwara, I., and Jawetz, E., *J. Infect. Dis.*, **124**, 255 (1971).
5 Becker, Y., and Zakay-Rones, Z., *Nature*, **222**, 851 (1969).
6 Becker, Y., *Israel J. Med. Sci.*, **8**, 1110 (1972).
7 Ben Ishai, Z., Heller, E., Goldblum, N., and Becker, Y., *Nature*, **224**, 29 (1969).
8 Wang, S. P., and Grayston, J. T., in *Trachoma and Related Disorders* (edit. by Nichols, R. L.), 322 (Excerpta Medica, Amsterdam, 1971).
9 Jawetz, E., and Hanna, L., *Proc. Soc. Exp. Biol. Med.*, **105**, 207 (1960).
10 Nabli, B., in *Trachoma and Related Disorders* (edit. by Nichols, R. L.), 34 (Excerpta Medica, Amsterdam, 1971).
11 Tribby, I. I. E., Friis, R. R., and Moulder, J. W., *J. Infect. Dis.*, **127**, 155 (1973).

Scrapie-like Antigen(s) in Ageing Tissues

Some of the morphological changes in the brain which are found both in the light and electron microscopes in the relatively young mouse or rat with scrapie are also present in the normal animal in old age[1]. These include enlargement and increased aurophilia of astroglia, presence of amyloid bodies, occurrence of closely orientated tubules within axis cylinders, and the occurrence of intranuclear inclusion bodies[2]. Recently, too, plaques resembling those found in senile brains have been reported in the scrapie mouse (H. Fraser and M. Bruce, demonstration at the Pathological Society of Great Britain and Ireland, July 1972). It would appear that the disease process has brought forward in time many of the features normally found in old age. Similar ageing changes have not, however, been reported in other organs, perhaps because no study has been specifically directed towards them.

Recently it has been found that brain or spleen from a scrapie mouse when injected into a guinea-pig results in a sensitization of the animal's lymphocytes which shows itself as a greater reaction to scrapie mouse brain presented as antigen than to normal mouse brain. This scrapie–normal difference (SND) can indeed be used as a means of rapid diagnosis of scrapie in the mouse[3]. Experiments have therefore been set up to determine whether the same difference can result from injection of tissues of old mice and humans. Furthermore, since our previous report it has been established that the increased SND we proposed as a rapid means of diagnosing scrapie can also be elicited by the injection of other tissues such as muscle, liver, and kidney from scrapie mice—in other words the new scrapie antigenic properties are not limited to brain and spleen.

Brain, spleen, liver or kidney in the form of 10^{-1} suspension in saline cleared by centrifugation for 10 min at 1,800*g* was prepared from mice of different ages and inoculated (0.1 ml intracutaneously: dorsum right foot) into adult Hartley guinea-pigs (500 g: both sexes) and their lymphocytic reactivity to scrapie mouse brain and spleen, compared with normal brain and spleen, estimated after 8 d. The mouse tissues used as inocula came from Webster-Swiss (male) animals between 27 d and 35 months of age. The guinea-pig lymphocytes were tested after 8 d by the macrophage electrophoretic migration (MEM) test described by Field and Caspary[4,5] of which full operational details together with an experimental protocol *in extenso* are given by Caspary and Field[6]. In principle the method depends upon the observation that when sensitized lymphocytes interact with antigen, a protein is liberated into the ambient medium with the property of causing normal guinea-pig macrophages to travel more slowly in an electric field. Normal macrophages can thus be used as an indicator system for lymphocyte-antigen interaction. If t_e = time of macrophage migration when antigen is present with lymphocytes and macrophages; t_c = control time, that is when no antigen is present; then $t_e > t_c$ and $t_e - t_c/t_c \times 100$ is a measure of lymphocyte sensitization. It is

these percentage slowing figures which are presented in the tables.

Response to scrapie material is always greater than to corresponding normal tissue. The latter indeed remains almost unchanged. The difference between response to scrapie and normal (SND) has been calculated in Table 1 where it is clear that in respect of both brain and spleen it increases with the age of the mouse from which the material was taken for injection into the guinea-pig. Indeed the SND comes into the range observed with tissue derived from a scrapie mouse when the injected tissue is from a normal animal of 28 months. This special antigenic property of old tissue is not limited to the nervous system. Liver and kidney also showed these antigenic properties as the animal became older.

In a second series of experiments formalin fixed human brain, spleen, heart, liver and kidney suspensions were made (10^{-1} in saline after washing out the formalin) from subjects of different ages ranging from 20 to 91 (supplied by Dr J. N. Corsellis). All were female. The causes of death were: age 20: cerebral and subarachnoid haemorrhage associated with an arteriovenous malformation of the brain; age 30: bronchopneumonia and encephalitis; age 52: fracture of base of skull leading to carotid-cavernous fistula; age 72: perforated duodenal ulcer with generalized peritonitis; age 91: femoral vein thrombosis embolism.

All patients died from acute disease and none had suffered from chronic disease. In each case 0.1 ml of the 10^{-1} suspension cleared by centrifugation at 1,800*g* for 10 min was injected intracutaneously on the dorsum of the right foot of Hartley guinea-pigs. Lymphocyte sensitization was measured as in the previous experiments.

It is apparent from Table 2 that SND increases with age for all the human tissues studied just as in the ageing mouse. Here, too, the increase is largely due to the greater response to scrapie tissue antigen when guinea-pigs have been injected with older material. The occurrence of greater SND with ageing is thus shared by mice and humans.

In a third series of experiments, blood lymphocytes from humans of different ages were tested for reactivity towards scrapie and normal brain and spleen. No age differences were found. This indicates that whilst new antigenic determinant(s), akin or identical with those appearing in the young mouse with scrapie, appear in old tissues, the autologous lymphocytes show no special sensitization to them. This does not mean, however, that the antigenic properties of the lymphocytes themselves (when injected into guinea-pigs) are not affected by age. Old lymphocytes in fact have the same antigens as do old tissues in general (see below). An analogous situation has been observed with respect to leukaemic lymphocytes which, whilst unable to react with encephalitogenic factor[4] or cancer basic protein, nevertheless possess cancer basic protein determinant(s) on their surface[7]. It is interesting that only a minor (but significant) rise in lymphocyte sensitization to encephalitogenic factor (EF) and cancer basic protein (CaBP) has been found to occur with ageing[8]. The new determinant(s) arising in old tissues are thus regarded as "self" but are recognized as different from those present in young tissues when presented to guinea-pigs.

It was of interest to find out if the new antigen(s) resembling scrapie antigen might be present in (or on) old lymphocytes. Accordingly 10^7 cells prepared from a normal girl of 16 and from an old lady of 91 (normal) were injected into guinea-pigs and found to produce SNDs of 0.9 (brain) and 0.7 (spleen) for the young person; and 6.2 and 5.8 for the old. Thus lymphocytes, too, exhibit the same antigenic changes as other old tissues so that a specimen of these cells offers a convenient "biopsy" in assessing ageing or for research purposes.

Finally, because it is generally thought that thymectomy in the neonatal mouse accelerates its clinical ageing, a short series of experiments has been carried out to determine whether scrapie-like antigenicity appears precociously in the tissues of the young mouse thymectomized at birth compared with a normal or sham operated animal. It is clear from Table 3 that following neonatal thymectomy antigen(s) of the scrapie-old

Table 1 Mouse Ageing

	Age (d)	Scrapie brain	Normal brain	Scrapie spleen	Normal spleen	SND Brain	SND Spleen
A	Brain injected						
	27	10.4	9.4	5.2	4.0	1.0	1.2
	42	9.1	7.9	6.0	4.7	1.2	1.3
	180	12.1	9.9	6.2	4.0	2.2	2.2
	203	12.4	9.9	7.0	4.4	2.5	2.6
	575	13.2	9.4	9.4	4.4	3.8	5.0
	850	13.8	9.7	9.1	4.3	4.1	4.8
	1,065	14.1	9.3	8.7	4.3	4.8	4.4
B	Spleen injected						
	27	5.0	3.8	10.1	8.9	1.2	1.2
	203	6.8	4.3	11.8	9.2	2.5	2.6
	575	9.1	4.1	13.6	9.1	5.0	4.5
	850	9.0	4.2	12.8	8.8	4.8	4.0
	880	9.2	4.2	14.1	9.4	5.0	4.7
	1,065	7.6	2.8	13.2	9.6	4.8	3.6
C	Liver injected						
	27	5.5	4.4	5.4	4.4	1.1	1.0
	203	6.9	4.4	6.7	4.4	2.5	2.3
	575	7.6	3.9	7.2	3.2	3.7	4.0
	850	7.9	3.9	7.5	2.9	4.0	4.6
	880	9.2	4.3	9.7	4.4	4.9	5.3
D	Kidney injected						
	27	5.3	4.4	5.2	4.2	0.9	1.0
	203	7.0	4.6	6.9	4.3	2.4	2.6
	575	7.7	4.0	7.0	3.4	3.7	3.6
	850	8.0	4.4	7.7	3.7	3.6	4.0

Fresh mouse tissues (0.1 ml: 10^{-1}) injected into guinea-pigs and lymphocyte sensitization measured after 8 d.

Table 2 Human Ageing

	Antigen				SND	
	Scrapie brain	Normal brain	Scrapie spleen	Normal spleen	Brain	Spleen
Age (yr)						
A Brain injected						
F, 20	10.8	9.7	5.4	4.5	1.1	0.9
F, 30	10.3	9.3	5.3	3.9	1.0	1.4
F, 52	12.9	9.1	9.4	5.8	3.8	3.6
F, 72	14.3	9.6	11.1	5.7	4.7	5.4
F, 91	13.5	9.2	10.9	6.0	4.3	4.9
B Spleen injected						
F, 30	5.3	4.3	10.4	9.8	1.0	0.6
F, 91	10.5	3.9	14.8	9.3	6.6	5.5
C Heart muscle injected						
F, 30	5.1	4.3	5.2	4.3	0.8	0.9
F, 91	9.0	4.2	8.4	4.1	4.8	4.3
D Liver injected						
F, 30	5.1	4.3	5.0	4.0	0.8	1.0
F, 91	10.2	4.5	10.3	4.4	5.7	5.9
E Kidney injected						
F, 30	5.2	4.2	5.0	4.2	1.0	0.8
F, 91	8.8	3.5	9.1	4.4	5.3	4.7

Formalin fixed human brain, spleen, heart, liver and kidney injected into guinea-pigs (0.1 ml: 10^{-1}) and lymphocyte sensitization measured after 8 d.

Table 3 Effect of Thymectomy on SND

	Antigen				SND	
	Scrapie brain	Normal brain	Scrapie spleen	Normal spleen	Brain	Spleen
(a) Brain injected						
Thymectomized	12.6	9.3	8.0	4.2	3.3	3.8
Sham (normal)	9.6	8.3	6.1	4.9	1.3	1.2
(b) Spleen injected						
Thymectomized	8.5	4.2	13.2	9.2	4.3	4.0
Sham (control)	—	—	—	—	—	—

Neonatally thymectomized animal aged 39 d: brain and spleen injected into guinea-pigs: lymphocyte sensitization measured after 8 d.

age type rapidly appear so that SND of the order ordinarily given by the normal mouse of about 12 months is found in an animal with a calendar age of only 39 d. Neonatal thymectomy has an extensively documented effect on the development of the lymphatic system, and immunological processes have been thought to be involved in the mechanism of ageing[9–12]. Recently[13] Fabris *et al.* have examined the possible importance of the thymus as a biological clock and of hormones for the ageing processes of the lymphoid system. They found thymus dependent cells important in preventing early ageing, a conclusion consistent with that from our own recent work. Different strains of mice show considerable variation in ageing changes[14], so it would be of interest to study the dependence of SND upon the various factors considered by Simms[15] as affecting the ageing process.

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1 Field, E. J., *D. Z. Nervenheilk.*, **192**, 26 (1967).
2 Field, E. J., and Peat, A., *Gerontologia*, **17**, 129 (1971).
3 Field, E. J., and Shenton, B. K., *Nature*, **240**, 104 (1972).
4 Field, E. J., and Caspary, E. A., *Lancet*, ii, 1337 (1970).
5 Field, E. J., and Caspary, E. A., *J. clin. Pathol.*, **24**, 179 (1971).
6 Caspary, E. A., and Field, E. J., *Brit. med. J.*, **2**, 613 (1971).
7 Dickinson, J. P., Caspary, E. A., and Field, E. J., *Brit. J. Cancer* (in the press).
8 Field, E. J., and Smith, J. K., *Gerontologia*, **18**, 185 (1972).
9 Burnett, F. M., *Brit. med. J.*, **2**, 720 (1959).
10 Walford, R. L., *J. Gerontol.*, **17**, 281 (1962).
11 Walford, R. L., *The Immunologic Theory of Ageing* (Munksgaard, Copenhagen, 1969).
12 Burch, P. R. J., *Growth, Disease and Ageing* (Oliver and Boyd, Edinburgh, 1968).
13 Fabris, N., Pierpaoli, W., and Sorkin, E., *Nature*, **240**, 557 (1972).
14 Thung, P. J., *Gerontologia*, **1**, 223 (1957).
15 Simms, H. S., *Pathology of Laboratory Rats and Mice* (edit. by Cotchin, E., and Roe, F. C.), 733 (Blackwell, Oxford, 1967).

Malignant Tumours of Liver and Lung in Rats fed Aminopyrine or Heptamethyleneimine together with Nitrite

SOME human cancer might be caused by nitrosamines formed in the gastrointestinal tract from nitrite in the food and secondary or tertiary amines ingested deliberately or incidentally[1–3]. This hypothesis was supported by experiments in which rats fed with nitrite and secondary amines developed the same type of tumours as after treatment with the respective nitrosamines[4].