Our data indicate significantly different effects of lithium on normal and CGL CFU-GM. The reason for this difference is unknown, but it is not surprising considering the multiple defects in the response to regulatory factors observed in CGL cells[16,17].

The hypothesis that the action of lithium that we observed on CFU-GM is not direct, but mediated by other cells is unlikely in view of the low number of cells plated; at this density no endogenous production of Colony Stimulating Activity (CSA) is detectable (15).

In conclusion, considering the caution required in view of the limitations of in vitro systems for studying in vivo phenomena, our data do not seem to support the hypothesis that lithium can enhance the proliferation of a silent leukemic clone. However, caution in the use of lithium to treat granulocytopenias is warranted until the relevance in humans of the late depletion of stem cells, observed in mouse continuous cultures of hemopoietic stem cells[7] is established.

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2 G. Rothstein, D. Clarkson, W. Larsen, B.I. Grosser and J.W. Athens, New Engl. J. Med. *298*, 178 (1978).
3 R.S. Stein, G. Hanson, S. Koethe and R. Hansen, Ann. intern. Med. *88*, 809 (1978).
4 Editorial, Lancet *2*, 626, 1980.
5 R.S. Stein, J.M. Flexner and S.E. Graber, Blood *54*, 636 (1979).
6 G. Tisman, V. Herbert and S. Rosenblatt, Br. J. Haemat. *24*, 767 (1973).
7 L.J. Levitt and P.J. Quesenberry, N. Engl. J. Med. *302*, 713 (1980).
8 W.G. Harker, G. Rothstein, D. Clarkson, J.W. Athens and J.L. Mac Farlane, Blood *49*, 263 (1979).
9 G. Tosato, J. Whang Peng, A.S. Levine and D.G. Poplack, Blood *52*, 1033 (1978).
10 W.P. Hammond and F. Appelbaum, New Engl. J. Med. *302*, 808 (1980).
11 R.S.T. Jim, Ann. intern. Med. *92*, 262 (1980).
12 P.E. Bille, M.K. Jensen, J.P.K. Jensen and J.C. Poulsen, Acta med. scand. *198*, 281 (1975).
13 D.L. Longo, New Engl. J. Med. *303*, 283 (1980).
14 M. Aglietta, G. Camussi and W. Piacibello, Exp. Hemat. *9*, 95 (1981).
15 B.L. Pike and W.A. Robinson, J. cell. Physiol. *76*, 77 (1970).
16 M. Aglietta, W. Piacibello and F. Gavosto, Cancer Res. *40*, 2507, 1980.
17 H.E. Broxmeyer and M.A.S. Moore, Biochim. biophys. Acta *516*, 129 (1978).

Pathological changes in inbred strains of mice following early thymectomy and irradiation[1]

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Summary. Mice subjected to thymectomy and irradiation were found to develop a range of pathological change in various organs. These changes were accompanied by antibodies to a variety of self-components. The pattern of pathological and autoimmune change was found to vary with the strain. This strain-related expression did not appear to be associated with the major histocompatibility complex (H-2).

Autoimmune disease presents a continuing problem both in clinical terms and also in understanding the basic mechanisms of aetiology and pathogenesis. Efforts to study the fundamental aspects of such diseases have to a large extent depended upon attempts to induce similar states in laboratory animals by immunization with appropriate self components and complex adjuvants[3,4]. However, such procedures often lead to failure or inconsistent reproduction of autoimmune changes which in any case tend to differ from the clinical condition in many basic respects.

Evidence accumulated over the last decade, indicating that thymus-derived cells and their products play an important role in the regulation of the immune response, including reactions of the autoimmune type, has enabled a different approach to be made to the development of relevant experimental models of autoimmunity[5,6]. In this study we describe the induction of a wide spectrum of pathological changes with some evidence for autoimmune origin in inbred mice strains, following interference with immune regulatory function by early thymectomy and irradiation without the requirement for autoimmunisation. This particular approach affords a new opportunity to investigate the basic pathogenic mechanisms underlying a number of autoimmune lesions in a variety of organs, and to investi-

Summary of changes in mice observed after early thymectomy (21 days of age) and sub-lethal irradiation (4 × 250 rad)

Strain	H-2 Type	No. of animals	Incidence of autoantibodies (%)					Incidence of changes in solid organs (%)		
			Erythrocytes	Nuclear components	Parietal cell*	Thyroid	Smooth muscle	Thyroid	Liver	Kidney
CBA/H	k	22	27	0	20	0	0	0	0	0
AKR	k	8	38	25	0	12	0	0	0	0
BALB/c	d	20	30	0	20	15	0	10	0	0
DBA/2	d	6	17	17	60	0	0	0	0	0
C57BL/10	b	14	21	8	0	0	7	0	28	14
SJL/J	s	15	0	34	20	7	6	7	33	20
SWR/J	q	9	0	11	20	22	0	11	0	0

* 5 animals per strain were tested. No changes were observed in normal control mice of the above strains with the exception of two mice of SJL/J (8%) and DBA/2 (7%) strains which had weak ANA.

gate their possible genetic associations, particularly with the major histocompatibility complex (MHC), which is now known to be associated with increased susceptibility to many clinical manifestations of autoimmunity[7-10].

Materials and methods. Mice. Various inbred mice were obtained initially from Dr J.B. Smith of John Curtin Medical School, Canberra, Australia. They were bred and maintained in our animal facility.

Thymectomy and irradiation. Mice were thymectomized at 21 days of age and 2 weeks later subjected to a series of sub-lethal doses of x-radiation (4×250 rad) at 14-day intervals. Mice were killed 2 months after the last irradiation and various organs collected.

Coomb's test. Anti-erythrocyte antibodies were detected by this test as described elsewhere[11].

Immunofluorescence tests. a) Antinuclear antibodies (ANA). Mouse liver or kidney and vero cells were used as a nuclear substrate to detect ANA. b) Antiparietal and anti-smooth muscle antibodies. These were detected by using mouse and rat stomach mucosae as a source of parietal cells and smooth muscle.

Tanned haemagglutination assays. This assay was performed to detect anti-thyroglobulin antibodies using rat thyroglobulin as a cross reacting antigen.

Results. Marked strain differences in pathological consequences were observed some 2 months following Tx-X treatment. Details of the resulting changes were as follows (table).

Anti-erythrocyte antibody development was the most common autoimmune change observed. AKR and BALB/c strain mice had the highest incidence of these antibodies (38% and 30% respectively). A moderate incidence was also observed in CBA/H and C57BL/10 strain mice.

Some of the anti-erythrocyte antibody-positive strains manifested changes in the haemopoietic system such as splenomegaly and reduced PCV. Variability was also observed in this respect in individuals in a particular strain.

Antinuclear antibodies (ANA) were also readily induced in several strains of mice. SJL/J and AKR strains of mice had high levels of ANA (34% and 25% respectively). Homogeneous, rim, and to a lesser extent diffuse speckled nuclear staining patterns were observed in these strains suggesting that there were antibodies to several nuclear antigens. A low incidence of ANA was detected in C57BL/10 and SWR/J mice.

Antibodies to smooth muscles were detected by immunofluorescence in a limited number of strains (C57BL/10 and SJL/J). Positive sera also gave an intense fluorescent staining pattern with glomerular basement membranes and blood vessel walls.

An intense immunofluorescent staining of parietal cells was observed in DBA/2 and CBA/H mice with an incidence of 60% and 20% respectively (fig. a). These cross-reacted with rat parietal cells but not with other cells from liver, kidneys and heart suggesting that they are tissue-specific but not species-specific. A moderate incidence (20%) of antiparietal cell antibodies was also detected in SWR/J, SJL/J and BALB/c mice but of lower intensity than the other positive strains.

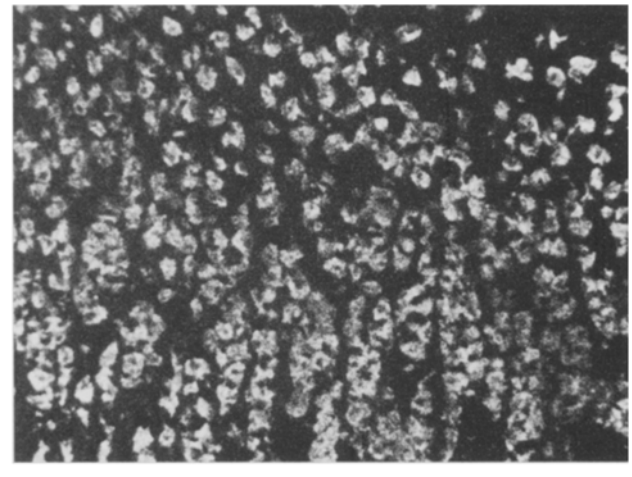
About 10% of Tx-X BALB/c and SWR/J mice developed mild lesions of the thyroid gland following treatment. The lesions were typically autoimmune in appearance and were characterized by a moderate infiltration of mononuclear cells together with a few plasma cells (fig. b). These histological changes were accompanied by antibodies to thyroglobulin. Although thyroid lesions were not evident, low levels of autoantibodies to thyroglobulin were also detected in AKR and SJL/J strains of mice.

SJL/J mice exhibited moderate to severe pathological changes in liver with an incidence of 33%. The lesions, predominantly situated in the hepatoportal triad, were characterized by mononuclear cell infiltration together with some plasma cells and neutrophils. Some degree of fibrosis and hypertrophy of bile ducts was also observed in these strains. Similarly, C57BL mice had a moderate incidence (28%) of liver change. Although severe destruction of hepatic tissue was not as apparent as in SJL/J mice, there were a number of foci of mononuclear infiltration scattered throughout the liver.

Severe glomerular changes were also observed in 20% of SJL/J and to a lesser extent in C57BL/10 mice. The characteristic feature of the lesion was an infiltration of mononuclear cells around the glomerulus and in some animals there was total glomerular destruction.

Thymectomy and irradiation did not induce any obvious histological changes in the brain, hypophysis, adrenals, intestines, or gall bladder in any strains so far examined. Although some of the strains appeared to have SLE-like syndromes no histological evidence of generalised vascular changes were seen.

Discussion. In this study using a limited range of H-2 haplotype strains, the development of a wide range of pathological changes following thymectomy and irradiation was observed. Most of these were accompanied by autoan-



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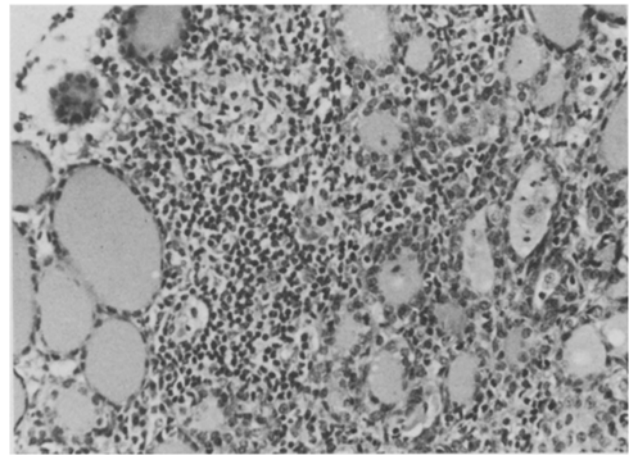

b

Representative changes following early thymectomy (21 days) and irradiation (4×250 rad) of mice. *a* Shows intense immunofluorescent staining of mouse parietal cells by autoantibodies in serum obtained from DBA/2 strain mice two months after final irradiation. ×86. *b* Shows the thyroid of a BALB/c strain mouse 2 months after final irradiation. Note the heavy mononuclear infiltration and destruction of follicles. H & E, ×86.

tibodies suggesting that there was an abrogation of self-tolerance maintained by the thymus or thymus-derived cells. Neonatal thymectomy alone has also been shown to induce or enhance autoimmunity in several systems[12-14]. This suggests that irradiation does not play a direct tissue-damaging role in the genesis of the lesions. This aspect has been extensively studied in a rat model of autoimmune thyroiditis in this laboratory, where it has been shown conclusively by shielding[6], lymphoid cell transfer[15] and also post-irradiation thyroid grafting[16] studies that irradiation does not directly cause or even potentiate the lesions observed. Furthermore, only the combination of early thymectomy and irradiation led to the development of severe lesions whilst either thymectomy or irradiation alone resulted in little if any change[6]. These findings also suggest that some of the consequences of severe irradiation previously ascribed to a direct damaging effect may be generated via autoimmune mechanisms.

Although the influence of genetic background on the susceptibility to autoimmune changes is clearly evident from the strain differences it was not possible to relate this to the major histocompatibility complex. It is likely that other non-MHC linked genes may be important in influencing the susceptibility in this particular situation. However, the H-2 basis of susceptibility to autoimmune diseases in this particular context needs further examination in other strains of mice with similar or differing H-2 haplotypes.

This approach should have many applications in studying basic autoimmune pathogenesis. For instance, it affords an opportunity of investigating the mechanisms responsible for a wide range of autoimmune phenomena, particularly those involving the thyroid, stomach and haemopoetic system. Furthermore, the availability of a wide variety of inbred congenic and mutant mouse strains will enable the relationship between the genetic make-up and a particular expression of the spectrum of autoimmunity to be examined in depth.

1 This study was partly supported by the National Health and Medical Research Council of Australia.
2 Author for correspondence and reprint requests.
3 E. Witebsky and N.R. Rose, J. Immun. *76*, 408 (1956).
4 P.Y. Paterson, Adv. Immun. *5*, 131 (1966).
5 P.J. Baker, P.W. Stashak, D.F. Amsbaugh, B.A. Prescott and R.F. Bach, J. Immun. *105*, 1581 (1970).
6 W.J. Penhale, A. Farmer, R.P. McKenna and W.J. Irvine, Clin. exp. Immun. *15*, 225 (1973).
7 P. Strastny, J. clin. Invest. *57*, 1148 (1976).
8 D. Bernoco and P.I. Terasaki, in: Genetic control on autoimmune diseases, p.3. Ed. N.R. Rose, P.E. Bigazzi and N.L. Warner. Elsevier/North-Holland, Amsterdam 1978.
9 P.J. Morris, H. Vaughan, B.D. Tait and I.R. Mackay, Aust. N.Z. J. Med. *7*, 616 (1977).
10 A. Svejgaard, P. Platz, L.P. Ryden, L.S. Nielsen and M. Thomsen, Transplant. Rev. *22*, 3 (1973).
11 K.O. Cox and J.J. Finlay-Jones, Br. J. exp. Path. *60*, 466 (1979).
12 J. Thivolet, J.C. Monier, J.P. Ruel and M.H. Richard, Nature *214*, 1134 (1967).
13 A. Kojima, Y. Tanaka-Kojima, T. Sakakura and Y. Nashizuka, Lab. Invest. *84*, 550 (1976).
14 W.J. Penhale, unpublished observations.
15 W.J. Penhale, W.J. Irvine, J.R. Inglis and A. Farmer, Clin. exp. Immun. *25*, 6 (1976).
16 S. Ansar Ahmed and W.J. Penhale, Clin. exp. Immun., in press (1981).

The silver-staining technique – a tool for characterizing lymphocyte populations in mammalian peripheral blood

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Summary. Nucleolus organizer regions (NORs) of the peripheral blood lymphocytes (PBL) of 9 mammalian species were analyzed by means of a silver-staining procedure. Species-specific NOR patterns were demonstrated. The number of NOR chromosomes was positively correlated with the nucleolar coefficient, and negatively correlated with the relative frequencies of uninucleolar cells in PBL interphase.

Silver-staining methods have been applied for the localization of nucleolus organizer regions (NORs) in metaphase and/or in interphase cells[2-5]. The classical works of Heitz[6] and McClintock[7] on plant material demonstrated that the sites of nucleolus formation at telophase occur at the NORs present on certain chromosomes. Heneen[4] confirmed, on mammalian monolayers, that the silver-staining observed at metaphase corresponds to that expressed at later stages of mitosis and during early interphase, and is nucleolar in nature. Beran and Pospichil[8], using toluidine blue staining, found species differing in their ratio of uninucleolar and multinucleolar lymphocytes and in the nucleolar coefficient (mean of silver-stained nucleoli per lymphocyte) in peripheral blood lymphocytes (PBL). PBL are highly heterogeneous, and most of them are believed to be highly differentiated (G_O-phase) and to divide seldom. However, most of them can be activated by both unspecific and specific antigens to re-enter the cell cycle and become cycling. In human PBL, an increase in silver-staining from 1 or 2 stained area(s) to several – up to 5 or more – individual, small areas has been reported after PHA stimulation[5].

The present investigation was aimed at determining the suitability of the silver-staining technique applied for detecting NOR variabilities in unstimulated PBL interphases, by assessing the relative frequencies of PBL with distinct silver-stained areas, and the nucleolar coefficient, and comparing them with the diploid number of NORs, in several mammalian species.

Material and methods. Peripheral blood was taken from unimmunized, healthy, adult individuals, 2 male and 2 female of each of the following species; man *(Homo sapiens)*, rabbit *(Oryctolagus cuniculus)*, horse *(Equus caballus)*, pig *(Sus scrofa)*, dog *(Canis familiaris)*, cat *(Felis catus)*, cattle *(Bos taurus)*, sheep *(Ovis aries)*, and goat *(Capra hircus)*. 10 ml of peripheral blood was centrifuged at $400 \times g$, and the buffy coat withdrawn, fixed with 3:1 methanol acetic acid for 10 min, then pipetted onto slides and air-cried. In later experiments a short, hypotonic treatment (37 °C, 5 min) of the buffy coat cells before fixation seemed further to improve the contrast of the deeply-stained nucleolar chromocenters against pale background structures that sometimes occurred in addition to them.